IN VITRO

VOLUME 36 NUMBER 3, PART II MARCH 2000

CELLULAR & DEVELOPMENTAL BIOLOGY

PROGRAM ISSUE

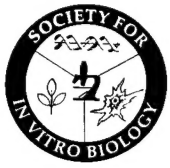
2000

World Congress on In Vitro Biology

June 10 - 15, 2000
San Diego, California

The Town & Country Resort

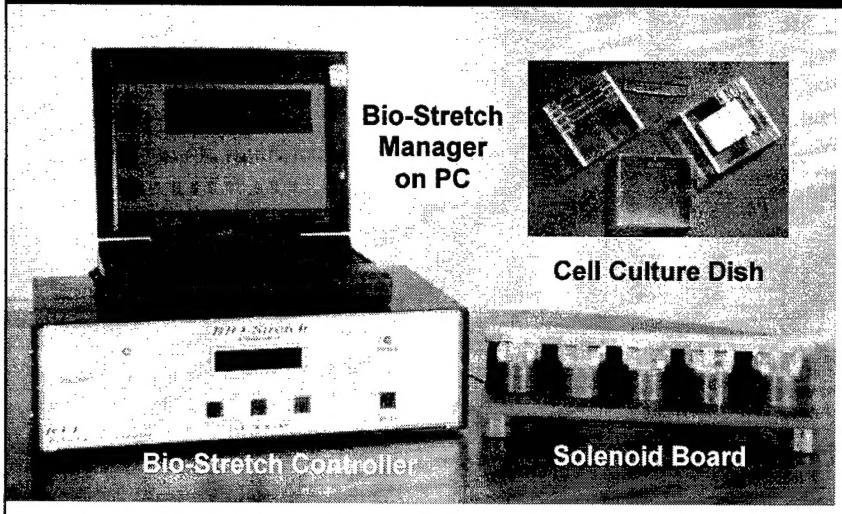
PROGRAM ISSUE



Journal of the Society for In Vitro Biology

Dynamically GROW cells/tissues, STRETCH cells/tissues, STUDY what happens to them !

BIO-STRETCH SYSTEM



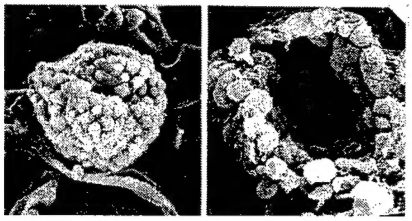
Features

- **Culture Cells/Tissues Dynamically**
- **Stretch 3D and 2D Cultured Cells**
- **Multiple Stretch Patterns (Forces)**
- **Regular and Irregular Stretch**
- **Allow Comparative Studies**
- **GUI Software under Windows**
- **Easy to use and operate**

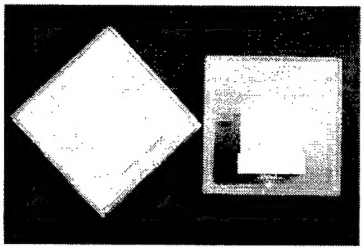
STRETCHING IS GOOD TO GROWING CELLS. Bio-Stretch Device is a novel computerized stretch instrument which applies user-defined static/variable duration cyclic stretch to growing cells in vitro. Bio-Stretch is an ideal selection for biomedical research field as it induces biochemical changes in response to applied stretch in cells derived from bone, cardiac, kidney, skeletal, muscle, lung, vascular vessels, skin, tendon, ligament, and cartilage that are normally subjected to tension, stretch or compression in vivo.

DO YOU KNOW? A basic Bio-Stretch System consists of a Bio-Stretch Controller, a Bio-Stretch Manager software running on PC, three (3) solenoid boards (each with 5 solenoids), and an IBM compatible PC (optional).

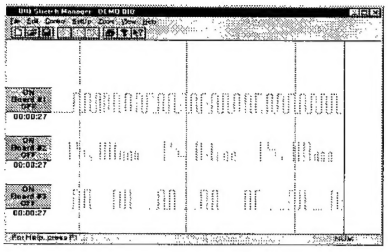
DO YOU KNOW? The Bio-Dish allows you to culture more than EIGHT (8) million cells on a Gelfoam sponge inside it.



Yes! Bio-Stretch allows the user to perform stretch on 3D cultured cells/tissues. Its special designed cell culture dishes provide an ideal platform for 3D or 2D cell culture.



Newly redesigned cell culture dish has more room for solution. It also increases the precision of measurement between experiments.



Bio-Stretch Manager allows users adjust factors such as burst, rest, cycle, strength, etc. which means full control of experimental process.

Visit Us at: http://www.iccttech.com

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Abstracts

AD_____

Award Number: DAMD17-00-1-0590

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PRINCIPAL INVESTIGATOR: John Harbell, Ph.D.

CONTRACTING ORGANIZATION: Society for In Vitro Biology
Largo, Maryland 20774

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IN VITRO PLANT
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In Vitro Cellular & Developmental Biology — Plant

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Aims and scope

In Vitro Cellular & Developmental Biology– Plant publishes peer-reviewed original research and reviews concerned with the latest developments and state-of-the-art research in plant cell and tissue culture and biotechnology from around the globe. Four issues cover cellular, molecular and developmental biology research using *in vitro* grown or maintained organs, tissues or cells derived from plants. Two special IAPTC&B issues deal with plant tissue culture, and molecular and cellular aspects of plant biotechnology. The IAPTC&B and SIVB maintain completely separate and independent International Editorial Review boards for their issues. From the start of the 2000 volume *In Vitro Cellular and Developmental Biology — Plant* will be available in print and on the Internet in Acrobat PDF and HTML formats.

Topics covered by the journal include:

- biotechnology/genetic transformation
- developmental biology/morphogenesis
- micropropagation
- functional genomics
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- metabolic engineering
- plant physiology
- cell biology
- somatic cell genetics
- secondary metabolism

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Saturday

7:00	Registration-Grand Ballroom Foyer
9:00-13:00	Educational Outreach Program K-12 for Teachers In Vitro Techniques for the Classroom UCSD Campus
9:00 – 17:00	Advanced Tissue Culture Workshop 9:00 – 12:00 Session A Sunrise 12:00 – 13:00 Lunch Break 13:00 -17:00 Session B Sunrise
12:00-13:30	10th International Conference on Invertebrate Cell and Tissue Culture Invertebrate Awards Forum Invertebrate Reception Pacific 4 & 5 12:00 – 12:30 Reception 12:30 – 13:30 Welcome and Awards Forum Keynote Address "Foundation for Achievement in the New Millennium"
13:30-15:30	Strategies for Culturing Cells from Fastidious Invertebrates Invertebrate Symposium Pacific 6 & 7
Poster Information	SUN. JUNE 11 10:00 – 21:00 MON. JUNE 12 10:00 – 21:00 TUES. JUNE 13 10:00 – 13:00 POSTER SESSION Posters mounted Saturday, June 10 15:00 – 17:00. Posters must be removed from Exhibit Hall by 14:00, June 13. Authors will be present at their posters the following days and times: Sat. June 10 All Authors 19:30 – 20:30 Sun. June 11 Even Authors 13:00 – 13:30 Mon. June 12 Odd Authors 13:00 – 13:30 Tues. June 13 All Authors 12:00 – 12:30
15:40-17:40	Invertebrate Hormones Invertebrate Symposium Pacific 6 & 7
Evening Events	2000 World Congress Opening Reception 19:00 Grand Ballroom

Sunday

7:00	Registration-Grand Ballroom Foyer
7:00–8:00	Publications Committee Pacific 4 Cell Culture Standardization Committee Meeting Pacific 5 Education Committee Meeting Pacific 6
8:00-10:00	Marine Organisms as Models for Biomedical Research Joint Vert/Invert/Tox Symposium Royal Palm 1 Advanced Physiologically Functional Foods for Prevention of Disease General Symposium Royal Palm 2 Cryopreservation for International Genetic Resource conservation in the 21st Century Plant Symposium Royal Palm 3 & 4
10:00	Coffee Break
10:00 – 15:00 Exhibits and Posters 10:30 - 12:30	Secondary Products Plant Contributed Paper Session Royal Palm 3 & 4 In Vitro Production of Recombinant Proteins and Viral Pesticides Joint Vert/Invert/Tox Symposium Royal Palm 1 NASA Biotechnology: Cell Science in Microgravity Vertebrate Symposium Royal Palm 2 Technology Transfer to Essential Crops in the Developing World Plant Workshop Royal Palm 5 & 6
10:00 – 15:00 Exhibits and Posters 12:30- 14:30	In Vitro Research for High School Students Education Symposium Dover Current Issues Facing Tissue Culture Collections in the New Millennium Vertebrate Workshop Sunrise
13:30 – 14:30	Transgenic Plants Plant Contributed Paper Session Royal Palm 3 & 4 Recombinant Protein Production in Insect Cells Invertebrate Contributed Paper Session Royal Palm 1
15:00-16:30	OPENING PLENARY SESSION Town and Country Room "Preservation of Endangered Plant, Animal and Insect Species"
Evening Events	Plenary Reception and Dinner San Diego Zoo Buses Depart by 18:00

Monday			Tuesday	
7:00		Registration-Grand Ballroom Foyer		
8:00-10:00		Mechanical Determinants of Cell Form and Function Vertebrate Symposium — Royal Palm 2 In Vitro Models to Study Cancer Vertebrate Symposium — Royal Palm 1 Phytoremediation-Removal & Detoxification of Environmental Contaminants by Plants Plant Symposium — Royal Palm 3 & 4 Micropropagation Plant Contributed Paper Session — Royal Palm 5 & 6 Marine Invertebrate Cell Culture Invertebrate Contributed Paper Session — Pacific 4 & 5	8:00-10:00	The New Era of Blood Cell Cultures: Breakthrough Techniques & Clinical Applications Vertebrate Symposium — Royal Palm 2 Fucntional Genomics and Proteomics in Plant Biology Plant Workshop — Royal Palm 3 & 4 The Role of In Vitro Techniques in the Preservation of Endangered Plant and Animal Species General Symposium — Royal Palm 5 & 6 Molecular Aspects of Plant Disease Plant Contributed Paper Session — Pacific 4 & 5 Insect Cell Culture Applications Invertebrate Contributed Paper Session — Pacific 6 & 7
10:00		Coffee Break		
10:00-15:00 Exhibits and Posters	10:30-12:30	Plant Transformation Plant Contributed Paper Session — Royal Palm 5 & 6 Tissue Engineering: From Cells to Organs Vertebrate Symposium — Royal Palm 2 In Vitro Biology of Tick-borne Diseases: Lyme Disease and Ehrliciosis Joint Vert/Invert/Tox Syposium — Royal Palm 1 Micropropagation of Tropical Plants Plant Symposium — Royal Palm 3 & 4	10:30-11:30	DISTINGUISHED PLENARY SESSION Town and Country Room "Advances in Understanding the Molecular, Genetic, and Protein Structure of Neurodegenerative Diseases"
	12:30-13:30	Exhibitors/SIVB Reception — Grand Ballroom Odd Poster Authors in Attendance	11:30-13:00	Distinguished Plenary Reception Grand Ballroom All Poster Authors in Attendance
13:30-15:30		Bioinformatics and Data Management Systems: Computational Genomics General Symposium — Royal Palm 2 Growth Factors in Differentiation and Carcinogenesis Vertebrate Symposium — Royal Palm 1 Cell Wall Biosynthesis: The Cell's Sixth Sense Plant Symposium — Royal Palm 3 & 4	13:30-15:30	Cell Death/Cell Cycle Control in Plants Plant Workshop — Royal Palm 3 & 4 Stem Cell Biotechnology Vertebrate Symposium — Royal Palm 5 & 6
			13:30-17:30	Molecular Biology of Fish Cells In Vitro Joint Vert/Invert/Tox Sympoium — Pacific 4 & 5
			14:00-15:30	In Vitro Models for the Study of Cancer Vert/Tox Contributed Paper Session — Royal Palm 2
15:30-17:30		In Vitro Models to Study Tissue Dynamics and Cell Interactions in Stratified Epithelium Cellular Toxicology Symposium — Royal Palm 2 Metabolic Engineering of Secondary Metabolism in Plants: Principles and Applications Plant Symposium — Royal Palm 3 & 4 The Challenge of In Vitro Collection and Field Collections for Tissue Culture Plant Workshop — Royal Palm 5 & 6	15:30-17:30	Neural Stem Cell Development Joint Vert/Invert/Tox Symposium — Royal Palm 1 Genes That Control Plant Development & Their Potential Use in Tissue Culture & Transformation Plant Workshop — Royal Palm 3 & 4

Wednesday

7:00	Registration-Grand Ballroom Foyer
8:00-10:00	Molecular Farming – Plant Biotechnology's Next Big Wave Plant Symposium — Royal Palm 3 & 4 Proteinases & Inhibitors in Angiogenesis Vertebrate Symposium — Royal Palm 2 Somatic Embryogenesis Plant Contributed Paper Session — Royal Palm 5 & 6 Insect Midgut Invertebrate Symposium — Royal Palm 1
8:00-12:30	(Invertebrate Session Coffee Break 10:30 – 11:00)
10:00	Coffee Break
10:30-12:30	New Technologies for Advancing Drug Development & Discovery Cellular Toxicology Symposium — Royal Palm 2 Alternative Markers for Plant Transformation Plant Workshop — Royal Palm 3 & 4
12:30-13:30	Characterization of Animal Cells: Viral Contamination and Authenticity Vertebrate Symposium — Royal Palm 2
13:00-14:00	Factors Affecting Cellular Growth and Differentiation I Vert/Tox Contributed Paper Session — Royal Palm 1 Factors Affecting Cellular Growth and Differentiation II Vert/Tox Contributed Paper Session — Royal Palm 2 Invertebrate Section Meeting 13:00 – 14:00 — TBA
14:30-16:30	The Revolution in Molecular and Cellular Biology Presents Unique Challenges and Opportunities for High Throughput Screening Application Cellular Toxicology Symposium — Royal Palm 1 – 2 Beyond Science: Impact of Agriculture Biotechnology on Production, Food, and Society Plant Symposium — Royal Palm 3 & 4
Evening Events	SIVB Business Meeting 17:00 – 18:00 — Royal Palm 5 & 6 Reception/Silent Auction 19:00 – 20:00 — Grand Ballroom Foyer Millennium Banquet 20:00 – 22:00 — Town and Country Room

Thursday

	Registration-Town and Country Foyer
8:00-12:30	Bt Transgenic Crops: Efficacy, Environmental Effects, and Resistance Management Joint Invertebrate/Plant Symposium — California (Coffee Break in Foyer from 10:15 – 10:45)
	2000 WORLD CONGRESS ADDITIONAL MEETINGS AND EVENTS
Friday Events, June 9	Program Committee Meeting 13:00 – 15:00 — Royal Palm 1
Saturday Events, June 10	SIVB Executive Board Meeting 8:00 – 12:00 — Dover 2000 Program Planning Committee Meeting 12:00 – 13:00 — Royal Palm 1 History Society Meeting 18:00 – 19:00 — Presidential Suite
Monday Events, June 12	Plant Section Business Meeting/Social 18:00 – 21:00 — Sunrise Vertebrate/Cellular Toxicology Section Meeting/Social 19:00 – 21:00 — Pacific Salon 4 & 5 Posters Remain Open 17:30 – 21:00 — Grand Ballroom
Tuesday Events, June 13	Development Committee Meeting 7:00 – 8:00 — Dover Student Affairs and Awards Committee Meeting 7:00 – 8:00 — Stratford Poster Breakdown and Removal 13:00 – 14:00 — Grand Ballroom
Wednesday Events	Membership Committee Meeting 7:00 – 8:00 — Pacific 4 Constitution and Bylaws Committee Meeting 13:30 – 14:30 — Dover

2000 World Congress on In Vitro Biology ~ Schedule of Functions

TIME	TYPE OF FUNCTION	ROOM
FRIDAY, JUNE 9		
17:00 – 21:00	SIVB Executive Board Meeting	Dover
SATURDAY, JUNE 10		
7:30– 19:30	Registration	Grand Ballroom Foyer
8:00- 12:00	SIVB Executive Board Meeting	Dover
9:00– 17:00	Educational Outreach Program K-12 for Teachers	USCD Campus
9:00–17:00	Advanced Tissue Culture Workshop	Sunrise
12:00- 13:00	2000 Program Planning Committee Meeting	Royal Palm 1
15:00– 17:00	Poster Set-up	Grand Ballroom
15:30- 17:30	IAPTC&B / SIVB Officers Meeting	Dover
18:00- 19:00	History Society Meeting	Presidential Suite
19:00- 21:00	Opening Reception (Poster Presentations 19:30 – 20:30)	Grand Ballroom
SUNDAY, JUNE 11		
7:00– 18:00	Registration	Grand Ballroom Foyer
7:00 – 8:00	Publications Committee Meeting	Pacific 4
7:00 – 8:00	Cell Culture Standardization Committee Meeting	Pacific 5
7:00 – 8:00	Education Committee Meeting	Pacific 6
10:00– 10:30	Coffee Break	Grand Ballroom
10:00– 15:00	**Exhibits and Posters**	Grand Ballroom
12:30– 13:30	In Vitro – Plant Editorial Board Meeting	Pacific 4
12:30– 14:30	Education Outreach K-12 Symposium	Dover
13:30– 14:30	CABI / SIVB Editorial Luncheon Meeting	Pacific 5
13:00 – 13:30	Even Poster Presentations	Grand Ballroom
17:30 – 18:00	Evening at the San Diego Zoo – Board Buses	Convention Foyer
18:00 - 21:00	Evening at the Zoo/Reception, Tour and Dinner	San Diego Zoo
MONDAY, JUNE 12		
7:00– 18:00	Registration	Grand Ballroom Foyer
7:00 – 8:00	Strategic Long-Range Planning Committee Meeting	Pacific 5
7:00 – 8:00	Laboratory Materials & Biosafety Committee Meeting	Pacific 6
7:00 – 8:00	Plant Program Committee Breakfast Meeting	Pacific 4
10:00 – 10:30	Coffee Break	Grand Ballroom
10:00 – 15:00	**Exhibits and Posters**	Grand Ballroom
13:00 – 13:30	Odd Poster Presentations	Grand Ballroom
12:30 – 13:30	Special Reception Sponsored by Exhibitors and SIVB	Grand Ballroom
17:30 – 21:00	Posters Remain Open	Grand Ballroom
18:00 – 21:00	Plant Section Business Meeting/Social	Sunrise
19:00 – 21:00	Vertebrate/Cellular Toxicology Section Meeting/Social	Pacific Salon 4-5
TUESDAY, JUNE 13		
7:00 – 18:00	Registration	Grand Ballroom Foyer
7:00 - 8:00	Development Committee Meeting	Dover
7:00 – 8:00	Student Affairs Committee Breakfast Meeting	Stratford
10:00– 10:30	Coffee Break	Grand Ballroom
11:30– 13:00	Plenary Reception (Poster Presentations 12:00 – 12:30)	Grand Ballroom
13:00– 14:00	Poster Breakdown and Removal	Grand Ballroom
WEDNESDAY, JUNE 14		
7:00 - 19:00	Registration	Grand Ballroom Foyer
7:00 – 8:00	Membership Committee Meeting	Pacific 4
10:00 – 10:30	Coffee Break	Grand Ballroom Foyer
13:00 – 14:00	Invertebrate Section Meeting	TBD
13:30 – 14:30	Constitution and Bylaws Committee Meeting	Dover
17:00 – 18:00	SIVB Business Meeting	Royal Palm 5-6
19:00 – 20:00	Reception / Silent Auction	Grand Ballroom Foyer
20:00 – 23:00	Millennium Dinner	Town & Country
THURSDAY, JUNE 15		
13:00 – 15:00	Program Committee Meeting	Royal Palm 1

Note: Additions and changes to functions will be posted on a bulletin board located in the registration area. Please check the bulletin board daily.

WORLD CONGRESS ON IN VITRO BIOLOGY
2000 Meeting of the Society for In Vitro Biology
June 10-15 • San Diego, California

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INTERNATIONAL 24-HOUR CLOCK

All sessions are scheduled using the international 24-hour click, in contrast to the United States custom of stating time in 12 hour increments: a.m. and p.m. The following chart is provided to assist registrants with the 24-hour clock notation.

24 hr	U.S.	24 hr	U.S.
13:00	1:00 pm	19:00	7:00 pm
14:00	2:00 pm	20:00	8:00 pm
15:00	3:00 pm	21:00	9:00 pm
16:00	4:00 pm	22:00	10:00 pm
17:00	5:00 pm	23:00	11:00 pm
18:00	6:00 pm	24:00	12:00 am

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SATURDAY, JUNE 10

8:00 – 19:00 Registration Grand Ballroom Foyer

8:00 – 12:00 SIVB EXECUTIVE BOARD MEETING Dover

EDUCATIONAL OUTREACH PROGRAM K-12

Conveners: Zuzana Zachar, PhD, Department of Biochemistry and Cell Biology
State University of New York at Stony Brook
Patricia Bossert, PhD, Northport High School Research Program, New York

9:00 – 13:00 In Vitro Techniques for the Classroom UCSD Campus

A "Hands-on" Workshop for High School Teachers

Presented by Society for In Vitro Biology and Cooperative Alliance of Research Teachers/Students, Northport High School, Stony Brook University, Cincinnati Zoo & Botanical Garden, Phytotechnology Laboratories

This educational program will introduce *in vitro* biology teaching modules that can be taken back to the classroom. This program will consist of three hands-on workshops (sessions A-C) and a symposium (Session D). The workshops will instruct participants in DNA isolation and polymerase chain reaction (PCR); collection of plant tissue for culture; and culture of plant cells to include use of specialized media and contamination control. The symposium, held as part of the 2000 World Congress, will focus on research and teaching strategies to mentor pre-college students in the field of *in vitro* biology.

Participation is strictly limited to workshop pre-registrants.

Faculty : ***Patricia Bossert, PhD,*** *Northport High School Research Program, New York*
Valerie Pence, PhD, *Ctr. for Research of Endangered Wildlife, Cincinnati Zoo & Botanical Garden*
Kenneth C. Torres, PhD, *Phytotechnology Laboratories, LLC*
Jennifer Visconti, *Science Department, Northport High School, New York*
Zuzana Zachar, PhD, *Department of Biochemistry and Cell Biology, State University of New York at Stony Brook*

ADVANCED TISSUE CULTURE WORKSHOP

Convener: Burt Lidgerding, PhD, Sheperd College

8:30 – 17:00 Cell and Tissue Culture: Foundations and Advanced Applications Sunrise

The Foundations and advanced applications tissue culture workshop will provide information about the basic requirements for cell culture. Focus of this workshop will be on the culture of animal cells. Attendees should be familiar with cell culture facilities and have a general understanding of aseptic techniques. The morning session will discuss media requirements, sources of cells, quality control of cells, and cell cryopreservation. The afternoon program will be devoted to topics related to the production of biological molecules from cells in culture. Theory and practical applications will be included in each lecture presentation. The workshop topics are designed to be useful to college faculty, graduate students, and research technicians. Participants will be given a course syllabus containing a list of contacts and resource materials for cell culture topics. One continuing education credit (CEU) may be obtained through the University of California, San Diego after successful completion of the workshop course.

Participation is limited to workshop pre-registrants.

Saturday, June 10

10TH INTERNATIONAL CONFERENCE ON INVERTEBRATE CELL AND TISSUE CULTURE

SPONSORS: AVENTIS, MERCK RESEARCH LABORATORIES, PRIMEDICA

Chair: **Cynthia L. Goodman, PhD, US Department of Agriculture, ARS, BCIRL**
Co-Chair: **Lehman Ellis, PhD, Our Lady of Holy Cross College**

INVERTEBRATE AWARDS FORUM AND RECEPTION

SPONSORS: AVENTIS, MERCK RESEARCH LABORATORIES, PRIMEDICA

Convener: Cynthia L. Goodman, PhD US Department of Agriculture, ARS, BCIRL

12:00 Reception
12:30 Welcome and Awards Forum: Pacific 4 & 5
Lifetime Achievement Awards:
James L. Vaughn, PhD, *US Department of Agriculture, ARS, IPL*
Jun Mitsuhashi, PhD, *Department of Bioscience, Tokyo University of Agriculture*

I-1 Keynote Address
Foundation for Achievement in the New Millennium
James L. Vaughn, PhD, *US Department of Agriculture, ARS, IPL*

(See abstracts page 12-A)

PLENARY SESSION

STRATEGIES FOR CULTURING CELLS FROM FASTIDIOUS INVERTEBRATES

SPONSORS: AVENTIS, MERCK RESEARCH LABORATORIES, PRIMEDICA

Conveners: Karl Maramorosch, PhD, Rutgers State University of New Jersey
Cynthia L. Goodman, PhD, US Department of Agriculture, ARS, BCIRL

13:30 – 15:30 Invertebrate Symposium Pacific 6 & 7
(See abstracts page 12-A)

A variety of continuously growing cell cultures have been established from a handful of insect species, primarily lepidopterous and dipteran pest insects. For the most part, the establishment of these cell lines has been driven by the desire to develop novel strategies for controlling homologous hosts. Unfortunately, the techniques used to develop cell lines from pest insects have not always been directly transferable to other invertebrates, especially marine invertebrates. The symposium will focus on these issues and describe new, innovative means of generating cell cultures that may lead to the initiation of cell lines from otherwise difficult-to-culture invertebrate cells.

13:30 Introduction (C.L. Goodman and K. Maramorosch)
13:40 I-2 Development of Highly Nutritive Culture Media
Jun Mitsuhashi, PhD, *Tokyo University of Agriculture*
14:10 I-3 Novel Techniques to Establish New Insect Cell Lines
Dwight E. Lynn, PhD, *US Department of Agriculture, ARS*

Saturday, June 10

14:40 I-4 Pantropic Retroviral Vectors for Gene Transfer into Invertebrate Cells
Jane C. Burns, PhD, *Department of Pediatrics, University of California, San Diego, School of Medicine*

15:00 – 1700 Poster Set up Grand Ballroom

15:10 – 15:40 Break for Invertebrate Conference Foyer

INVERTEBRATE HORMONES

Convener: Renee Wagner, PhD, US Department of Agriculture, ARS, BCIRL

15:40 – 17:40 Invertebrate Symposium Pacific 6 & 7
(See abstracts page 13-A)

This session involves the discussion of invertebrate hormones and their relationship to tissue, organ, or cell culture. Hormones may be synthesized by particular cells or tissues, or they may regulate the development, activity or function of the cultures of interest. The synthesis, release, target and activity of invertebrate hormones is a complex process, which may involve a cascade of events and several factors or second messengers. Hormone synthesis and release is not always representative of the entire insect system. The ability to look at a particular part of this process *in vitro*, without the interference of other modulators and modes of regulation, is a useful tool to describe the simplest units of a complex process. In addition, such systems may be useful in defining the activity of the hormone and in screening compounds for use as insect control agents, or as supplements to rearing media for beneficial insects. This symposium compares *in vivo* and *in vitro* activities of invertebrate organs, tissues or cell cultures; the synthesis and/or effects of hormones on these systems; and the comparison of hormone regulation and activities in different invertebrate tissues and cell lines.

15:40 I-5 Slow and Fast Regulation of Corpora Allata in a Heteropteran Insect
Magdalena Hodkova, PhD, *Institute Of Entomology, Czech Academy Of Sciences*

16:10 I-6 Metamorphosis During the Passage of Drosophilia Imaginal Disc Cell Lines
Martin J. Milner, PhD, *University Of St Andrews*

16:40 I-7 Juvenile Hormone Promotes the Maintenance of Lamellipodia in a Lepidopteran Cell Line, and Mimics the Effects of Signaling by Lysophosphatidic acid and Exogenous Phospholipase
Susanne D. Dyby, *US Department of Agriculture, ARS*

17:10 I-8 Analysis of Transcriptional Regulation by Ecdysteroids in Ecdysone Sensititve Cultured Cell Line Derived from the Silkworm, *Bombyx mori*
Shuichiro Tomita, *National Institute of Sericultural and Entomological Science*

2000 WORLD CONGRESS OPENING RECEPTION

19:00 – 21:00 Grand Ballroom

Saturday, June 10
All Poster Authors will be present
19:30 –20:30
(See list of posters on pages)

SUNDAY, JUNE 11

7:00 – 18:00 Registration Grand Ballroom Foyer

MARINE ORGANISMS AS MODELS FOR BIOMEDICAL RESEARCH

SPONSOR: HARBOR BRANCH OCEANOGRAPHIC INSTITUTE, INC.

Convener: Shirley A. Pomponi, PhD, Division of Biomedical Marine Research, Harbor Branch Oceanographic Institution, Inc.

8:00 – 10:00 Joint Vertebrate/Invertebrate/Toxicology Symposium Royal Palm 1

(See abstracts page 3-A to 4-A)

Many fundamental processes at the cellular and molecular level are conserved throughout evolution. Because of their specific adaptations to the marine environment, marine organisms have been unique and useful models for gaining a better understanding of analogous processes in humans. They are models for immune system function, cell cycle, fluid and ion transport, and neural functions. Marine organism models are used to study human disease processes, such as immune disorders, cancer, glaucoma, diabetes, and cystic fibrosis. The purpose of this workshop is to highlight some novel approaches to the development, use, and biomedical applications of in vitro marine models.

8:00 Introduction (S.A. Pomponi)

8:15 JS-6 Principles Learned from How Marine Organisms Osmoregulate: Applications to Biomedical Research
Joan D. Ferraris, PhD, *Laboratory of Kidney and Electrolyte Metabolism, National Institutes of Health*

8:45 JS-7 The Egg and I: Unique Insights from Studies on Embryos of Marine Organisms
David Epel, PhD, *Stanford University, Hopkins Marine Station*

9:15 JS-8 Dynamic Modulation of Neurons and Networks: Lessons from Lobsters and Crab Neurons
Eve Marder, PhD, *Department of Biology, Brandeis University*

9:30 JS-9 Biosilicification in Sponges and Its Application for *In Vitro* Synthesis of Polysilxanes
Katsuhiko Shimizu, PhD, *Marine Biotechnology Center, University, Santa Barbara*

ADVANCED PHYSIOLOGICALLY FUNCTIONAL FOODS FOR PREVENTION OF DISEASE

SPONSOR: JAPANESE ASSOCIATION OF ANIMAL CELL TECHNOGY

Conveners: Shuichi Kaminogawa, PhD, Department of Applied Biological Chemistry, University of Tokyo
Sanetaka Shirahata, PhD, School of Genetic Resources Technology, Kyushu University

8:00 – 10:00 General Symposium Royal Palm 2

(See abstracts page 2-A to 3-A, 11A)

Various food components significantly affect the body regulation systems to include: neural, immune, circulation, and hormonal regulation. New types of physiologically functional foods, designed for prevention of various diseases, are expected to decrease the incidence of diseases in the 21st century. This symposium introduces the research on intestinal tract immunology, food allergy and the design of anti-allergy foods *via* regulation of T cell functions. The wide range of peptides derived from food proteins are known to regulate body functions. Some active oxygen peptides have been associated with various lifestyle and/or aging-related

diseases such as cancer, diabetes, arteriosclerosis, and Alzheimer's disease. Research data suggests that the daily intake of anti-oxidative plant foods can reduce and/or prevent the effects of aging and disease. A new field of research on anti-oxidative water, which can scavenge active oxygen species in cultured animal cells, will be introduced. Anti-oxidative water has been shown to cause reversible telomere shortening in cancer cells and suppresses growth of cancer cells via. Anti-oxidative water also exhibits insulin-like activity to promote sugar intake into muscle and adipocytes, suggesting its preventative effect in diabetes.

8:00 Introduction (S. Kaminogawa and S. Shirahata)

8:05 JS-1 Functional Foods for Prevention of Allergies
Shuichi Kaminogawa, PhD, *Department of Applied Biological Chemistry, University of Tokyo*

8:25 JS-2 Regulation of Permeability in Intestinal Epithelium by Food Components
Makoto Shimizu, PhD, *Department of Applied Biological Chemistry University of Tokyo*

8:45 JS-3 Functional Peptides Derived from Proteins in Food
Masaaki Yosikawa, PhD, *Research Institute for Food Science, Kyoto University*

9:05 JS-4 Anti-oxidative Plant Foods for Disease Prevention
Toshihiko Osawa, PhD, *School of Bioagricultural Sciences, Nagoya University*

9:25 JS-5 Prospect on the Development of Physiologically Functional Foods
Kazuki Shinohara, PhD, *National Food Research Institute, Ministry of Agriculture, Forest, and Fisheries of Japan*

9:45 JS-44 Suppression of Cancer and Diabetes by Anti-oxidative Water
Sanetaka Shirahata, PhD, *School of Genetic Resources Technology, Kyushu University*

CRYOPRESERVATION FOR INTERNATIONAL GENETIC RESOURCE CONSERVATION IN THE 21ST CENTURY

SPONSORS: AVENTIS, CELLFOR, INC.

Conveners: Ali Golmirzaie, PhD, College of Agriculture, University of Arkansas
David Cyr, PhD, Silvagen Inc.
Barbara M. Reed, PhD, US Department of Agriculture, ARS

8:00 – 10:00 Plant Symposium Royal Palm 3 & 4
(See abstracts page 16-A to 17-A)

Cryopreservation is often looked upon as the storage form of the future. Advances in the past ten years have transformed theoretical techniques into viable storage protocols and the first clonal germplasm collections are now stored in liquid nitrogen. This symposium will explore aspects of cryopreservation for storage of clonal germplasm from a wide range of plant genera. An overview of requirements, challenges, and techniques for clonal germplasm conservation will be followed by discussion of specific advances in the storage of root and tuber crops, forest trees, and fruit and nut crops.

8:00 Introduction (A. Golmirzaie, D. Cyr, and B.M. Reed)

8:15 P-1 Cryopreserved Storage of Clonal Germplasm: The Promise is Fulfilled
Barbara M. Reed, PhD, *US Department of Agriculture, ARS, National Clonal Germplasm Repository*

8:35 P-2 Advances in Cryostorage for Root and Tuber Crops
Ali Golmirzaie, PhD, *College of Agriculture, University of Arkansas*

8:55 P-3 Cryopreservation as an Integral Part of Forest Genetics Programs
David Cyr, PhD, *Silvagen, BC Research and Innovation*

9:15 P-4 Recent Advantages in Cryopreservation of Fruit Germplasm
Carmine Damiano, PhD, *Fruit Tree Research Institute*

9:35 P-5 Newly Developed Encapsulation-Dehydration Protocol for Plant Cryopreservation of In Vitro – Grown Apices
Akira Sakai, PhD, *Hokkaido University*

Sunday, June 11

10:00 – 10:30 Coffee Break Grand Ballroom

10:00 – 15:00 Exhibits and Posters Grand Ballroom

SECONDARY PRODUCTS

Moderator: Mary Ann Lila Smith, PhD, University of Illinois

10:30 – 12:00 Plant Contributed Paper Session Royal Palm 3 & 4
(See abstracts page 39-A to 40-A)

10:30 P-1001 Optimized Metabolic Flux Distributions in Catharanthus roseus Cultures
Xuefang Huang, *University of Manchester Institute of Science and Technology, C. Cenkci, and F. Mavituna*

10:45 P-1002 Production of Pilocarpine by Pilocarpus pennatifolius Cultures
Chi Wai Tang, *University of Manchester Institute of Science and Technology, and F. Mavituna*

11:00 P-1003 Strategies for In Vitro Taxol Production by Taxus spp.
Eman Zalat, *University of Manchester Institute of Science and Technology, C.W. Tang, and F. Mavituna*

11:15 P-1004 Bioflavonoid Production in Suspension Cultures Expedites Recovery of a Complex Series of Condensed Tannins
Mary Ann Lila Smith, *University of Illinois, F.E. Kandil, and D.S. Seigler*

11:30 P-1005 Evaluation of Different Methods for Cryopreservation of Taxus Media Cell Suspension and Its Secondary Metabolite Profile
Tina Liao, *The University of British Columbia, N. Stoynov, J.P. Kutney, and E.A. Polishchuk*

IN VITRO PRODUCTION OF RECOMBINANT PROTEINS AND VIRAL PESTICIDES

CO-SPONSORS: GLAXO WELLCOME , HYCLONE

Convener: Dr.-Ing. Holger Hübner, Bioprocess Engineering, Berlin University of Technology

10:30 – 12:40 Joint Vertebrate/Invertebrate/Toxicology Symposium Royal Palm 1
(See abstracts page 4-A)

Insect cell cultures are frequently used for the production of recombinant proteins and viral pesticides. Although both products are different, similar technical and biological problems are encountered to yield a product of sufficient quality and quantity. This symposium will emphasize current developments to increase quantity and quality of recombinant proteins. Technical methods will demonstrate the production and influence of cultivation conditions on glycoprotein processing. The data on viral pesticide production will focus on scale-up techniques. The most current solutions to the problems related to the cultivation of insect cells in larger bioreactor systems will be presented, as well as experiences from field-tests with in vitro-derived baculovirus pesticides.

10:30 Introduction (H. Hübner)

10:35 JS-10 Key Features in the Exploitation of Insect Cells for the Production of Baculovirus and Recombinant Proteins
Just M. Vlak, PhD, *Laboratory of Virology, Wageningen Agricultural University*

11:00 JS-11 On Earth and in Space: Insect Cell Glycosylation Pathway
H. Alan Wood, PhD, *Boyce Thompson Institute at Cornell*

11:25 JS-12 In Vitro Production of Baculoviruses for Pest Control: From Insect to Formulated Bioinsecticide
Rolf CL Marteijn, *Educational Institute of Technology and Nutrition, Wageningen University*

11:50 JS-13 Production of Recombinant Proteins in High-Density Cultures of Insect Cells
William Barnett, PhD, *HyClone Laboratories*

12:15 JS-14 In Vitro Large Scale Production of Viral Pesticides
Stefan Weiss, DSc, *Biotechnology Consulting*

NASA BIOTECHNOLOGY: CELL SCIENCE IN MICROGRAVITY
SPONSOR: NASA –JOHNSON SPACE CENTER

Conveners: J. Millburn Jessup, MD, University of Texas Health Science Center, San Antonio
Neal R. Pellis, PhD, Cellular Biotechnology Program, NASA - Johnson Space Center

10:30 – 12:30 Vertebrate Symposium Royal Palm 2
(See abstracts page 24-A)

NASA has developed technology for propagating cells in altered gravity environments. These techniques are tools and models to understand basic cell response to gravity, and to use this environment to engineer tissue constructs for research and biomedical applications. The presentations in this symposium will advance new concepts in the cellular response to microgravity and the nature of tissue constructs from ground-based and space bioreactors. The investigators will present their results to date with cultures both in unit and microgravity.

10:30 Introduction (J. M. Jessup and N.R. Pellis)

10:45 V-1 Microgravity Studies of Cells and Tissues
Gordana Vunjak-Novakovic, PhD, *Massachusetts Institute of Technology, Cambridge*

11:05 V-2 Signal Transduction Mechanisms Involved in the 3-D Assembly and Neuroendocrine Differentiation of PC12 Cells Cultured in RWV Bioreactors
Peter I. Lelkes, PhD, *Department Of Medicine, University of Wisconsin Medical School, Madison*

11:25 V-3 Oxidative Stress, Metastasis, and Liver Function in RWV Reactors
J. Millburn Jessup, MD, *Department of Surgery, University of Texas Health Science Center, San Antonio*

11:45 V-4 Apoptosis in Human Peripheral Blood Lymphocytes in Modeled Microgravity
Diana Risin, MD, PhD, *Biotechnology Program, Wyle Laboratories – Life Sciences Systems & Services, Houston*

12:05 V-5 Erythropoiesis Regulators in Simulated Microgravity
Arthur J. Sytkowski, MD, *Director Laboratory of Cell & Molecular Biology, Beth Israel Deaconess Medical Center, Boston*

TECHNOLOGY TRANSFER TO ESSENTIAL CROPS IN THE DEVELOPING WORLD
SPONSOR: MONSANTO

Convener: Swapan K. Datta, PhD, International Rice Research Institute (IRRI)

10:30 – 12:30 Plant Workshop Royal Palm 5& 6
(See abstracts page 31-A)

It is estimated that 60% more plant foods will be required for the developing countries by the year 2020. Constraints on food production include less land and water, and a decreased agricultural workforce. New technologies have emerged with potential promise to solve the problems of food supply in the developing countries. The right crop plant technology with strategic guidelines needs to be implemented with partnership of the national agricultural programs. This symposium will highlights: potential key food crop technologies and how they can be used; national and international food crop boundaries; International Plant Research (IPR) sharing; and risks/benefits in deployment of new products.

10:30 Introduction (S.K. Datta)
10:45 W-1 Designer Crops for Better Nutrition in Developing Countries
Asis Datta, PhD, *Jawarhal Nehru University*
11:15 W-2 Transfer of Transgenic Rice for IRRI to Asian Countries for Evaluation and Breeding
Swapan K. Datta, PhD, *International Rice Research Institute (IRRI)*
11:45 W-3 The Relevance of Ag-Biotechnology for Developing Countries Revisited
Anatole Krattinger, PhD, *Executive Director, ISAAA*

IN VITRO RESEARCH FOR HIGH SCHOOL STUDENTS

SPONSOR: D-SQUARD BIOTECHNOLOGIES

Convener: Sarwan K. Dhir, PhD, Fort Valley State College

12:30 – 14:30 Education Symposium Dover

(See abstracts page 53-A to 55-A)

This symposium will demonstrate some of the possibilities that arise when mentoring high school students as they explore a variety of in vitro research projects. Speakers include educators, mentors, and students. Mentors will discuss approaches to facilitating the research efforts of pre-college students. Students will give presentations of their research projects.

12:30 Introduction: Elizabeth J. Roemer, State University of New York at Stony Brook and Society for In Vitro Biology Education Committee
12:45 E-2003 LIGASE Science Education Program: Teacher Training and In-house Research Programs
Zuzanna Zachar, PhD, *Department of Biochemistry and Cell Biology, State University of New York at Stony Brook*
12:55 E-2011 Mentoring High School Students in a Research Setting
Sarwan K. Dhir, PhD, *Fort Valley State University*
13:05 E-2004 Investigation of Evolutionary Relationships Among Mammals Using the Polymerase Chain Reaction
Team DNA - Paul Frake, Youngmie Han, and Lindsey Paluska, *Northport High School*
13:25 E-2009 Introduction of Vaccine Genes in Tomato
Melissa Shah, *Warner Robins High School*
13:45 E-2008 Propagation of Daylily by Conventional and Non-conventional Methods
Tiffany Swann, *Houston County High School*
14:05 Discussion

CURRENT ISSUES FACING TISSUE CULTURE COLLECTIONS IN THE NEW MILLENNIUM

SPONSOR: AMERICAN TYPE CULTURE COLLECTION

Hosts: Cell Culture and Standardization Committee, Laboratory Materials and Biosafety Committee

Moderator: Yvonne Reid, PhD, American Type Culture Collection

12:30 – 14:30 Vertebrate Workshop Sunrise

The need for human cell lines has increased with worldwide advances in genetic mapping, gene expression, and gene therapy. These advances have highlighted the need to have a system to authenticate and identify well-characterized cell lines free of microbial contaminants. Just as important to this issue is the use of cells as animal model systems and the regulatory issues facing the handling and transfer of these materials. The Cell Culture Standardization Committee (CCSC) in conjunction with the Laboratory Materials and Biosafety Committee (LMBC) will host a panel of international experts to discuss these issues. These

international experts will address issues pertaining to animal alternatives, cell line misidentification, microbial contamination and regulatory issues. This workshop will present an opportunity for effective discussions between the Scientists (the donor and user of the biological material) and the Collections (distributor of biological material). Workshop outcome will be the understanding of issues and methods to make available the highest quality, authenticated and well-characterized biological material to the scientific community.

Panelists:

Animal Alternatives
Tadao Ohno, PhD, *Director, RIKEN Gene Bank and Head Riken Cell Bank, RIKEN (Institute of Physical and Chemical Research)*
Cell Line Misidentification
Hans Drexler, MD, PhD, *Director, Department of Human and Animal Cell Cultures, DSMZ, German Collection of Microorganisms and Cell Cultures Microbial Contamination*
Microbial Contamination
Glyn N. Stacey, PhD, *Head of Cell Biology and Imaging, Division of Virology, National Institute for Biological Standards and Control*
Regulatory Issues
Frank Simione, *Regulatory Affairs, American Type Culture Collection*

TRANSGENIC PLANTS

Moderator: Mark C. Jordan, PhD, Agriculture and Agri-Food Canada

13:30 – 14:30 — Plant Contributed Paper Session — Royal Palm 3 & 4
(See abstracts page 47-A)

13:30 P-1033 Development of Transgenic Peanut (Arachis hypogaea L.) Plants Producing an Edible Vaccine Against Cholera
Jacquelyn R. Jackson, *Tuskegee University, M. Egnin, H. Mason, and C. S. Prakash*

13:45 P-1034 Durum Wheat with Altered High Molecular Weight Glutenin Gene
Mark C. Jordan, *Agriculture and Agri-Food Canada, S. Cloutier, C. Rampitsch, and N. Ames*

14:00 P-1035 Transgenic Cyclamen persicum Mill., Produced from Etiolated Hypocotyls, Stably Express the gusA Reporter Gene in Petals, Scapes, Leaf Laminae, Petioles and Corms, 27 Months After Infection with Agrobacterium tumefaciens
M.R. Boase, *New Zealand Institute for Crop and Food Research Limited, G. B. Spiller, and T.A. Peters*

14:15 P-1037 Comparison of Visual Screening, Herbicide and Antibiotic Selection of Transgenic Oat Plants
A.R. Carlson, *University of Wisconsin, and H.F. Kaeppler*

RECOMBINANT PROTEIN PRODUCTION IN INSECT CELLS

Moderator: Amy Wang, PhD, Aventis CropScience

13:30 – 14:30 — Invertebrate Contributed Paper Session — Royal Palm 1
(See abstracts page 36-A)

13:30 I-1001 Design of an Efficient Medium for Insect Cell Growth and Recombinant Protein Production
Laertis Ikonomou, *Catholic University of Louvain, G. Bastin, Y.-J. Schneider, and S.N. Agathos*

13:50 I-1002 Comparison of Immobilization Methods for Insect Cells
Laertis Ikonomou, *Catholic University of Louvain, J.-C. Drugmand, G. Bastin, Y.-J. Schneider, and S.N. Agathos*

14:10 I-1003 Expression of Recombinant Endostatin in Stably Transformed *Drosophila melanogaster* S2 Cells
In Sik Chung, *Kyung Hee University, J.H. Park, K.H. Chang, J.M. Lee, and I. S. Hwang*

Sunday, June 11
Even Numbered Poster Authors will be present
13:00pm –13:30pm
(See list of posters on pages)

OPENING PLENARY SESSION
SPONSOR: MONSANTO

Convener: Maud Hinchee, PhD, Monsanto Company
Sandra L. Schneider, DrPH, Research and Clinical Laboratory Systems

15:00 – 16:30 Distinguished Plenary Session Town & Country Room

Preservation of Endangered Plant, Animal, and Insect Species
(See abstract page 1-A)

15:00 Introduction: Sandra L. Schneider, DrPH, World Congress Program Chair
Opening Remarks: Delia Bethell, PhD, BioSeparations, Inc. and President, Society for In Vitro Biology

PS-1 Distinguished Plenary Speaker: ***Peter Raven, PhD,*** *Director Missouri Botanical Gardens, Engelmann Professor of Biology, Washington University and President's Committee of Advisors on Science and Technology*

18:00 – 21:00 Plenary Reception and Dinner San Diego Zoo
CO-SPONSOR: MONSANTO

MONDAY, JUNE 12

7:00 – 19:00 Registration Grand Foyer

MECHANICAL DETERMINANTS OF CELL FORM AND FUNCTION

SPONSOR: BECTON DICKINSON

Convener: Elizabeth J. Roemer, State University of New York at Stony Brook

8:00 – 10:00 Vertebrate Symposium Royal Palm 2

(See abstracts page 24-A to 25-A)

The complexity of living cells and tissues makes it difficult to appreciate the full significance of individual molecular components when they are studied in isolation. The importance of architecture and mechanics must be recognized in order to understand signal integration and biochemical control. Mechanical stresses applied at the tissue level can result in structural rearrangements at the cell and molecular level. Force is an important component in tissue and cell function, in processes ranging from the contraction of muscles to the alignment of chromosomes at the metaphase plate. At the cellular level, forces acting on the adhesive contacts of cells with extracellular matrix contribute significantly to cell shape, viability, signal transduction and motility. An integrated view of cell regulation incorporating mechanics and structure as well as chemistry has gained increasing importance. This session will explore the role of mechanical forces in cell and tissue function.

8:00 Introduction (E. Roemer)

8:15 V-6 The Mechanics of Cell Regulation
Donald Ingber, MD, PhD, *Department of Pathology, Harvard Medical School and Children's Hospital, Boston*

8:45 V-7 Cytoskeleton Matrix Linkages in Motility: Effects of Force, Src. and Position
Michael Sheetz, PhD, *Department of Cell Biology, Duke University Medical Center*

9:15 V-8 Laminar Flow Transduces an Atheroprotective Signal: Inhibiting TNF Alpha Activation of JNK Requires the ERL1/2 Pathway in Endothelial Cells
Bradford C. Berk, MD, PhD, *Department of Cardiology, University of Rochester Medical Center*

IN VITRO MODELS TO STUDY CANCER

CO- SPONSORS: AVON, WYETH-AYERST, INTERNATIONAL FOUNDATION FOR ETHICAL RESEARCH

Convener: Gertrude Buehring, PhD, University of California, Berkeley

8:00 – 10:00 Vertebrate Symposium Royal Palm 1

(See abstracts page 25-A)

In vitro systems are crucial to studying human cancer due to the ethics of direct experimentation with humans. The interaction between malignant and nonmalignant cells is an important determinant of tumor progression in vivo. Components of this interaction can be more precisely identified and explored using in vitro systems where variables are easily manipulated. This symposium will focus on research using culture systems with two different cell types as models for via interactions of cancer cells.

8:00 Introduction (G. Buehring)

8:15 V-9 Individualizing Cancer Chemotherapy by Tumor Histoculture
Robert M. Hoffman, PhD, *Anticancer, Inc.*

8:45 V-10 Stromal: Epithelial Interactions Modulating Invasion of Human Papillomavirus-transformed Keratinocytes
Joel Palesfsky, MD, *School of Medicine, University of California, San Francisco*

9:15 V-11 Human Myoepithelial Cells Exert Multiple Suppressive Effects on Breast Carcinoma Proliferation, Invasion and Angiogenesis
Sanford H. Barsky, MD, *University California, Los Angeles, School of Medicine*

PHYTOREMEDIATION – REMOVAL AND DETOXIFICATION OF ENVIRONMENTAL CONTAMINANTS BY PLANTS

SPONSOR: UST, INC.

Convener: Clayton Rugh, PhD, Department of Crop and Soil Sciences, Michigan State University

8:00 – 10:00 Plant Symposium Royal Palm 3 & 4
(See abstracts page 17-A)

This symposium will be a comprehensive survey of research using naturally occurring and genetically engineered plants in the cleanup of environmental pollutants. The natural physiological and biochemical capabilities for uptake and degradation of both heavy metal and organic contaminants by plants will be addressed. The utility of plant biotechnology for phytoremediation will be demonstrated for enhanced and novel traits conferred to transgenic plants. Progress will be shown for a new biotechnology discipline to develop laboratory model systems and pilot studies toward initial field trials

8:00 Introduction (C. Rugh)

8:15 P-6 Development of Transgenic Plants for Ecotoxic Mercury Degradation and Removal
Clayton Rugh, PhD, *Michigan State University, East Lansing*

8:40 P-7 Uptake and Degradation of Chlorinated Hydrocarbon Solvents By Plants
Lee Newman, PhD, *University of Washington, Seattle*

9:05 P-8 Physiology, Biochemistry and Molecular Biology of Metal Hyperaccumulation in Plants
David E. Salt, PhD, *North Arizona University, Flagstaff*

9:30 P-9 Defusing the Environment; Engineering Plants to Degrade Explosives
Neil C. Bruce, PhD, *University of Cambridge*

MICROPROPAGATION

Moderator: Maureen Fitch, PhD, Pacific Basin Agriculture Research Center, US Department of Agriculture, ARS

8:00 – 10:00 Plant Contributed Paper Session Royal Palm 5 & 6
(See abstracts page 40-A to 42-A)

8:00 P-1006 Bulb Growth and Stem Foundation in Lily Bulbets Regenerated *In Vitro*
Merel M Langens-Gerrits, *Bulb Research Centre, A.F. Croes, and G.J. de Klerk*

8:15 P-1007 Direct Shoot Regeneration and Microtuberization in Wild Cyclamen persicum Mill. Using Seedling Tissue
M. Al-Majathoub, *Jordan University of Science and Technology, and N.S. Karam*

8:30 P-1008 Selection of Plants for Phytoremediation
A. Gerth, *Bioplanta Gmbh, and A. Böhler*

8:45 P-1009 Growth and Yield of Clonally Propagated Papayas Compared to Multiple-Seedling Transplants
Maureen M. Fitch, *Pacific Basin Agriculture Research Center, US Department of Agriculture, ARS, T. Leong, L. Akashi, S. Ferreira, and P. Moore*

9:00 P-1010 Vegetative and Fruiting Comparison of Tissue Culture-Derived and Conventionally Propagated Date Palm (Phoenix Dactylifera L.) cv. Barhi Trees
Abdelrahman S. Al-Wasel, *King Saud University*

9:15 P-1011 Micropropagation of the Threatened Medicinal Plant Allium wallichii (Kunth) in Nepal
P. Malla, *University of Vienna, and S. Malla*

9:30 P-1012 Conservation of Virus-free Sweet Potato of Four Cultivars in Three Northeast Locations in México
H. Gutierrez M., *Facultade De Agronomia, Universidad Autónoma de Nuevo León, C.G.S. Valdés L., and L.A. Moreno A.*

9:45 P-1013 Plant Genetic Instability Detected by Microsatellites-Primers
X. J. Leroy, *Institut des Sciences Agro-Alimentaires et du Monde Rural, K. Leon, and M. Branchard*

MARINE INVERTEBRATE CELL CULTURE

Moderator: Lehman Ellis, PhD, Our Lady of Holy Cross College

8:00 – 10:00 Invertebrate Contributed Paper Session (See abstracts page 36-A to 37-A) Pacific 4 & 5

8:00 I-1004 Gene Transfer to Cells of the Eastern Oyster
John T. Buchanan, *Louisiana State University, T. Cheng, J.F.La Peyre, R.K. Cooper, and T.R. Tiersch*

8:25 I-1005 Pantropic Retroviral Vectors Mediate Foreign Gene Expression in Shrimp (*Penaeus stylirostris*)
H. Shike, *University of California, San Diego, School of Medicine, C. Shimizu, K.R. Klimpel, and J.C. Burns*

8:50 I-1006 Infection of Primary Cultured Cells from Two Oyster Species by Pantropic Retroviral Vectors
V. Boulo, *DRIM, IFREMER-CNRS, J.D. Moore, C. Shimizu, C.S. Friedman, and J.C. Burns*

9:15 I-1007 Medium Constituents Promoting the Attachment and Spreading of Cells of Eastern Oysters
J.F. La Peyre, *Louisiana State University, Y. Li, and J.T. Buchanan*

9:40 I-1008 Development of a Culture Medium for Cells of the Eastern Oyster, *Crassostrea virginica*
J.F. La Peyre, *Louisiana State University, and Y. Li*

10:00 – 10:30 Coffee Break Grand Ballroom

10:00 – 15:00 Exhibits and Posters Grand Ballroom

PLANT TRANSFORMATION

Moderator: Harold Trick, PhD, Kansas State University

10:30 – 12:30 Plant Contributed Paper Session (See abstracts page 42-A to 43-A) Royal Palm 5 & 6

10:30 P-1014 Induction of a Filamentous Form of Agrobacterium
J.J. Finer, *Ohio State University, K.R. Finer, K.M. Larkin, and B.J. Martin*

10:45 P-1015 Use of fluorescence in situ hybridization for mapping of transgenes and screening of homozygous plants in transgenic barley (Hordeum vulgare L.)
Hae-Woon Choi, *University of California, Berkeley, P.G. Lemaux, and M.-J. Cho*

11:00 P-1016 Development of Efficient Transformation Systems for Monocotyledonous Crop Species Using Highly Regenerative, Green Tissues
***Myeong-Je Cho,** University of California, Berkeley*

11:15 P-1017 Improvement of MAT (Multi-Auto-Transformation) Vector System for Generating Marker-Free Transgenic Plants
***H. Ebinuma,** Nippon Paper Industries Co., Ltd., K. Sugita, E. Matsunaga S. Endo, K. Yamada, and A. Kawaoka*

11:30 P-1018 A Novel MADS-box Gene is Required for Shoot Bud Induction in Excised Leaf Cultures of Paulownia kawakamii
***P.P. Kumar,** The National University of Singapore, and A.P. Prakash*

11:45 P-1019 Visualizing Green Fluorescent Protein
***D.J. Gray,** University of Florida, Gainesville, N.J. Barnett, M.M. Van Aman, D.P. Weaver, K.T. Kelley, S. Jayasankar, and Z.J. Li*

TISSUE ENGINEERING: FROM CELLS TO ORGANS

SPONSOR: BIOWHITTAKER CORPORATION

Convener: Gordana Vunjak-Novakovic, PhD, Massachusetts Institute of Technology

10:30 – 12:30 Vertebrate Symposium Royal Palm 2

(See abstracts page 26-A)

Engineering tissues by culturing cells on three-dimensional biodegradable polymers is a new approach to replace tissues and organs lost to disease or trauma. Ideally, cells within engineered tissues must exhibit the appropriate pattern of gene expression, and tissues must develop with appropriate structure. In addition, synthetic analogs to native extracellular matrix (ECM) created using biodegradable polymers are required to provide a structural template for tissue formation, and to convey chemical and mechanical regulatory signals to cells in engineered tissues. This symposium will: review recent efforts to control structure and function in engineered tissue using synthetic polymer matrices with properly designed mechanical properties and degradation rates; regulation of gene expression and tissue microstructure; the use of tissue culture bioreactors to (a) establish spatially uniform distribution of cells on polymer scaffolds, (b) maintain physiological concentrations of chemical species, (c) provide efficient mass transfer and physical stimuli to the growing tissue, and (d) carry out experimental and modeling studies of tissue development; the paradigms of functional engineered tissues expected to have a major clinical impact. Methods and technologies currently in use in clinical trials, which involve the creation of complex organ structures and the use of injectable materials, will be discussed.. Future directions in Tissue Engineering, including advances for fetal intervention, will be presented and discussed.

10:30 Introduction (G. Vunjak-Novakovic)

10:45 V-12 Synthetic Extracellular Matrices for Tissue Engineering
***David Mooney, PhD,** Department Biomedical Engineering, University of Michigan*

11:15 V-13 Bioreactor Cultivation Engineered Cartilage and Cardiac Muscle
***Gordana Vunjak-Novakovic, PhD,** Massachusetts Institute of Technology*

11:45 V-14 Current Clinical Trends and Changing Concepts in Tissue Engineering
***Anthony Atala, MD, PhD,** Harvard Medical School*

IN VITRO BIOLOGY OF TICK-BORNE DISEASES: LYME DISEASE AND EHRLICHIOSIS

SPONSOR: US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND

Conveners: Tim Kurtti, PhD, Department of Entomology, University of Minnesota
Jorge Benach, PhD, State University of New York at Stony Brook

10:30 – 12:30 Joint Vertebrate/Invertebrate/Toxicology Symposium Royal Palm 1
(See abstracts page 4-A to 5-A)

Tick borne pathogens live in two different, yet overlapping worlds. The *borellia* species, *Borrelia burgdorferi*, causes Lyme disease and the rickettsial *Ehrlichia* and *Anaplasma* species cause ehrlichiosis and anaplasmosis respectively. These pathogens of humans and domestic animals possess physiological and pathogenic mechanisms that enable them to infect and replicate in two quite divergent hosts, the mammal and the vector tick. Vertebrate and invertebrate *in vitro* systems have been developed to study replication and pathogenic mechanisms of these pathogens. This symposium will present the *in vitro* biology of tick borne pathogens in mammalian and tick cell culture. The changes in the physical and chemical environment that induce phenotypic changes in spirochetes and rickettsiae will be covered. Changes that are germane to the success of the pathogen and to our development of diagnostic protocols and vaccines for the prevention of tick borne diseases will also be discussed.

10:30 Introduction (T. Kurtti and J. Benach)

10:40 JS-15 Acquisition of Host Proteases by *Borrelia*
Jorge Benach, PhD, *Department of Pathology, State University of New York at Stony Brook*

11:05 JS-16 Investigating the Pathogenesis of Granulocytic Ehrlichiosis Using Neutrophils and Myeloid Cell Lines
J. Stephen Dumler, MD, *Department of Pathology, Division of Medical Microbiology, Johns Hopkins Univeristy*

11:30 JS-17 Tick Cell Culture: New approaches for *Anaplasma*
Katherine M. Kocan, PhD, *Department Of Anatomy, Pathology and Pharmacology, University of Oklahoma*

11:55 JS-18 Lyme Disease Spirochetes at the Tick and Mammalian Host Interface: Physiological and Immunological Consequences
Marygorret Obonyo, PhD, *Cell and Molecular Medicine, University of California, Riverside*

MICROPROPAGATION OF TROPICAL PLANTS

SPONSOR: TWYFORD PLANT LABORATORIES, NOVARTIS

Conveners: M.N. Normah, PhD, Universiti Kebangsaan, Malaysia
A. Abdelnour-Esquivel, PhD, Costa Rica Institute of Technology

10:30 – 12:30 Plant Symposium Royal Palm 3 & 4
(See abstracts page 18-A)

Many advances in micropropagation have been cited for temperate ornamentals, agronomic and forest species. However, progress in micropropagation of tropical species has been quite limited. This symposium will feature some work on tropical species which includes chayote, citrus, orchids and forest species. The use of micropropagation for propagation and conservation of these species will be highlighted.

10:30 Introduction (M.N. Normah and A. Abdelnour-Esquivel)

10:45 P-10 Micropropagation, In Vitro Conservation, and Characterization of Chayote (Sechium edule)
A. Abdelnour-Esquivel, PhD, *Costa Rica Institute of Technology*

11:10 P-11 *In Vitro* Shoot Regeneration of Several Citrus Species
M. N. Normah, PhD, *University Kebangsaan*

11:35 P-12 In Vitro Propagation of Tropical Forest Trees
Enny Sudarmonowati, PhD, *Indonesian Institute of Sciences*
12:00 P-13 Studies on *Amorphophallus titanum* Tissue Culture
Irawati, PhD, *Hebarium Bogoriensis*

12:30 – 13:30 **Reception in Exhibit Hall** Grand Ballroom
Exhibitors/SIVB Reception

Monday, June 12
Odd Poster Authors will be present
13:00pm – 13:30pm
(See list of posters on pages)

BIOINFORMATICS AND DATA MANAGEMENT SYSTEMS:
COMPUTATIONAL GENOMICS
SPONSORS: SUN MICROSYSTEMS, INC., INCYTE GENOME SYSTEMS, INC.
CO-SPONSOR: AVENTIS CROPSCIENCE

Conveners: Ray Shillito, PhD, Aventis CropScience
Sandra L. Schneider, DrPH, Research and Clinical Laboratory Systems

13:30 – 15:30 General Symposium Royal Palm 2
(See abstracts page 5-A to 6-A)

The rate of DNA sequencing is constantly increasing and handling the accumulating data is a major challenge. In order to be of use, the sequence information must be linked via databases to phenotypic characteristics which may reveal their function. It is essential that any organization that aims to develop new products *via* bioinformatics be able to develop and maintain these immense integrated databases. Such computational resources must not only hold the data, but allow researchers to perform complex queries, find information about specific portions of genes, and organize the information. Considerable time and effort is currently being devoted to the up-dating and engineering of such databases, and to the search engines that retrieve useful information from them. This symposium will present advances in high-speed DNA and protein database searches using leading edge computational techniques.

13:30 Introduction (S.L. Schneider and R. Shillito)
13:40 JS-19 Bioinformatics in the New Millennium
Darrell O. Ricke, PhD, *Novartis Agricultural Discovery Institute, Inc.*
14:10 JS-20 Informatics for High-Throughput Functional Genomics
Craig Liddell, PhD, *Paradigm Genetics*
14:40 JS-21 Gene to Screen: Genomics, Screening, and Information Management
Rick K. Kochhar, PhD, *Sphinx Pharmaceuticals and Eli Lilly and Company*
15:10 Discussion

GROWTH FACTORS IN DIFFERENTIATION AND CARCINOGENESIS

SPONSORS: THE JAPANESE TISSUE CULTURE ASSOCIATION, JAPAN TOBACCO, INC.

Convener: Miho Furue, DDS, PhD, Kanagawa Dental College

13:30 – 15:30 | Vertebrate Symposium | Royal Palm 1

(See abstracts page 26-A to 27-A)

Growth factors have multiple biological functions and are considered to play key roles in cell development and homeostasis, tissue repair, and carcinogenesis. The identification of the specific role of growth factors in these events may lead to development of new therapies for many diseases. This symposium will discuss the key roles of growth factors in differentiation, wound repair, and carcinogenesis and the novel application of growth factor(s) to clinical therapies.

13:30 Introduction (M. Furue)

Keynote Speaker:
Biological Functions and In Vitro Applications of Growth Factors
Yoshisuke Nishi, PhD, *Japan Tobacco, Inc.*

14:00 V-15 Wild Type-KGFR2-IIIb/KGFR Gene Inhibits Growth and Differentiation/Apoptosis in Salivary Gland Tumors In Vitro and In Vivo
Yan Zhang, DDS, PhD, *Hiroshima University School of Dentistry*

14:20 V-16 The Hepatocyte Growth Factor and Urokinase-type Plasminogen Activator in Lung Injury and Repair
Kimiko Takahashi, PhD, *Tokyo Medical University*

14:40 V-17 Autocrine Growth Factor and Estrogen-Independence in Human Breast Cancer Cells
Ginette Serrero, PhD, *University of Maryland School of Pharmacy and University of Maryland Marlene and Stewart Greenbaum Cancer Center*

15:00 V-18 Activin Plays an Important Role in Morphogenesis of Salivary Gland Cells
Miho Furue, DDS, PhD, *Kanagawa Dental College*

CELL WALL BIOSYNTHESIS: THE CELL'S 6TH SENSE

SPONSORS: MONSANTO, THE SCOTTS COMPANY

Convener: David Ellis, PhD, British Columbia Research Inc., Forest Biotechnology Center

13:30 – 15:30 | Plant Symposium | Royal Palm 3 & 4

(See abstracts page 19A)

The cell wall, a crucial component of all plant cells, affects numerous aspects of plant development. Until recently, our understanding of the biosynthesis of this complex structure, formed outside the plasma membrane, has been greatly lacking. This symposium will present recent advances in our understanding of cell wall biosynthesis. Studies will include: the development and utilization of in vitro systems to study cell wall and cotton fiber formation; molecular biology of cellulose synthesis; and the manipulation of cellulose binding domains to influence plant growth.

13:30 Introduction (D. Ellis)

13:40 P-14 Tracheary Element Formation: A Molecular Analysis
Maureen McCann, PhD, *John Innis Centre, Norwich Research Park*

14:15 P-15 Recent Update in Cellulose Biosynthesis and Structure
Malcohm Brown, PhD, *Department of Botany, The University of Texas, Austin*

14:50 P-16 Exciting World of Plant Cellulose Synthases
Chandrashekhar P. Joshi, PhD, *Michigan Technical University, Houghton*

IN VITRO TISSUE MODELS TO STUDY TISSUE DYNAMICS AND CELL INTERACTIONS IN STRATIFIED EPITHELIUM

SPONSORS: PROCTER AND GAMBLE, US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND

Conveners: Jonathan Garlick, DDS, PhD, State University of New York at Stony Brook
John Harbell, PhD, Institute for In Vitro Sciences, Inc.

15:30 – 17:30 | Cellular Toxicology Symposium | Royal Palm 2

(See abstracts page 22-A)

Cell-cell interactions play an important role in the regulation of tissue homeostasis. In particular, dynamic and reciprocal interactions between constituents of stratified epithelia have been shown to modulate the growth, differentiation and repair of this tissue. This interplay is based on three distinct processes: 1) the production of soluble factors with autocrine and paracrine properties; 2) cell-matrix interactions; and 3) signaling by direct cell contact. The development of in vitro tissue models, such as human skin equivalents generated with keratinocytes, fibroblasts, melanocytes, endothelium, mast cells and other cells, has opened new avenues to study these interactions. This symposium will discuss recent insights into the interactions between these cells types using these in vitro tissue models. In particular, emphasis will be placed on cell contact-mediated melanocyte-keratinocyte cross-talk, modulation of cytokine and growth factor production through keratinocyte-fibroblast paracrine networks and control of keratinocyte phenotype through interactions with neighboring keratinocytes. The implications of these findings in understanding tissue dynamics in health and disease will be discussed.

15:30 Introduction (J. Garlick and J. Harbell)

15:15 T-1 Epithelial-Mesenchymal Interactions in Normal Tissue Homeostasis and Early Neoplastic Progression of Skin Equivalents
Jonathan Garlick, DDS, PhD, *State University of New York at Stony Brook*

15: 45 T-2 Double Paracrine Regulation of Keratinocyte Growth and Differentiation by Mesenchymal Factors
Norbert Fusenig, PhD, *German Cancer Research Center, Heidelberg*

16:15 T-3 Biology of Melanocytes and Melanoma in Human Skin Reconstructs
Carola Berking, PhD, *Wistar Institute, Philadelphia*

METABOLIC ENGINEERING OF SECONDARY METABOLISM IN PLANTS: PRINCIPLES AND APPLICATIONS

SPONSOR: DUPONT

Convener: Tony Kinney, PhD, DuPont AgBiotech

15:30 – 17:30 | Plant Symposium | Royal Palm 3 & 4

(See abstracts page 19-A to 20-A)

The next generation of transgenic plants will be more resistant to environmental stress and pathogen infection due to the understanding the regulation of metabolic pathways. New crop plants, produced from secondary metabolic pathway are being developed. Unlike primary metabolic pathways, which are usually in equilibrium and often share metabolic flux control, secondary pathways have single rate-limiting steps. During the secondary metabolic pathway, modification in a single gene can result in changes of the final crop product. These techniques have resulted in successful storage wax production in commercial oilseed crops. This symposium will address state-of-the-art for engineering "secondary" pathways and recent advances in understanding of pathway regulation to produce new storage crop products.

15:30 Introduction (T. Kinney)
15:45 P-17 Understanding the Dynamics of Secondary Metabolic Pathways in Plants
William Gruissem, PhD, *University of California, Berkley*
16:10 P-18 Metabolic Engineering of the Phenylpropanoid Pathway for Plant and Animal Health
Richard. Dixon, PhD, *Noble Institute*
16:35 P-19 Engineering of Plant Secondary Metabolism for Human Nutrition and Health
Brian McGonigle, *Dupont Nutrition and Health*
17:00 P-20 Producing Novel Seed Oils in Transgenic Plants
James Metz, PhD, *Calgene/Monsanto*

THE CHALLENGE OF IN VITRO COLLECTION AND FIELD COLLECTIONS FOR TISSUE CULTURE

SPONSOR: UST, INC.

Moderator: Valerie Pence, PhD, Center for Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden

15:30 – 17:30 Plant Workshop Royal Palm 5 & 6

(See abstracts page 31-A to 32-A)

Field collected materials pose particular challenges for plant tissue culture. Methods of in vitro collection, or IVC, offer the potential for initiating tissue cultures in the field, providing ease of transport and minimal disturbance to the plant *in situ*. However, the culture of field collected tissues, whether by IVC or in the laboratory, is often hindered by higher rates of contamination than those encountered with greenhouse- or laboratory-grown materials. This workshop will discuss the potential and challenges of IVC and will examine the problem of contamination in field collected materials and some possible approaches to overcoming this challenge.

15:30 Introduction (V. Pence)
15:45 W-4 Field Collection of Woody and Herbacious Explants for In Vitro Culture
Michael H. Renfroe, PhD, *Department of Biology, James Madison University*
16:10 W-5 In Vitro Collection Techniques for Leaf and Bud Tissues
Valerie C. Pence, PhD, *Center For Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden*
16:35 W-6 Foraging Ascomycetes as Contaminants of Plant Tissue Cultures: Fungal Endophytes in Leaves and Stems
George Carroll, PhD, *Department of Biology, University of Oregon*
17:00 W-7 Disinfection of Field Collected Material Using Plant Preservative Mixure (PPM)
Assaf Guri, PhD, *Plant Cell Technology, Inc.*

TUESDAY, JUNE 13

7:00 - 19:00 Registration Grand Foyer

THE NEW ERA OF BLOOD CELL CULTURES: BREAKTHROUGH TECHNIQUES & CLINICAL APPLICATIONS

SPONSOR: THE JAPANESE TISSUE CULTURE ASSOCIATION

Conveners: Tadao Ohno, PhD, The Institute of Physical and Chemical Research (RIKEN)
Masayoshi Namba, MD, PhD, Institute of Molecular and Cellular Biology, Okayama University Medical School

8:00 – 10:00 Vertebrate Symposium Royal Palm 2

(See abstracts page 27-A to 28-A)

Culture of human hematopoietic stem cells and blood cells has entered into a new epoch-making stage. This symposium will present the latest innovations in hematopoietic stem cell culture including human bone marrow models; interaction of stem cells with endothelial cells; and clinical application of human cytotoxic T lymphocytes for tumor therapy.

8:00 Introduction (T. Ohno and M. Namba)

8:15 V-19 Human Hematopoietic Stem Cell Cultures
Tatsutoshi Nakahata, MD, PhD, *Institute of Medical Science, Department of Clinical Oncology, University of Kyoto*

8:40 V-20 Interaction between Hematopoietic Stem Cells and Endothelial Cells
Toshio Suda, MD, PhD*, Department of Cell Differentiation, Kumamoto University*

9:05 V-21 Multilineal Blood Cell Differentiation in a Human Bone Marrow Model
J.H. David Wu, PhD, *Departments of Chemical, Microbiology, Immunology and Biomedical Engineering, University of Rochester*

9:30 V-22 Human Cytotoxic T Lymphocyte Culture and Clinical Application in Tumor Therapy
Tadao Ohno, PhD, *Gene and Cell Bank, The Institute of Physical and Chemical Research (Riken)*

FUNCTIONAL GENOMICS AND PROTEOMICS IN PLANT BIOLOGY

SPONSOR: UST, INC.

Convener: Todd Jones, PhD, DuPont AgBiotech

8:00 – 10:00 Plant Workshop Royal Palm 3 & 4

(See abstracts page 32-A)

Genomics programs, both public and private, have produced an enormous amount of DNA sequence information. While a portion of the sequence information is immediately useful, the assignment of function to the majority of the sequences remains problematic. Several strategies have been developed to approach this problem. One such strategy is to examine the differences in gene expression under varying conditions leading to the creation of a gene expression profiles. These profiles can then be "mined" to identify those genes that respond to a particular treatment. This symposium will present alternate systems for expression profiling and the respective advantages of each profile treatment. Alternatively, functional proteomics, the identification and characterization of proteins in biological samples, is beginning to have an impact on plant biology. The issues related to proteomic programs will be discussed in relations to implications for crop improvement.

8:00 Introduction (T. Jones)
8:15 W-8 Bead-Based Genomics at Lynx
Ben Bowen, PhD, *Lynx Therapeutics, Inc.*
8:45 W-9 Expression Profiling of Plant Genomes Using GeneCalling™ Technology
Oswald R. Crasta, PhD, *Agricultural Genomics, Curagen Corporation*
9:15 W-10 Proteomics for Crop Improvement
Guru Rao, PhD, *Pioneer Hi-Bred International, Inc.*

THE ROLE OF IN VITRO TECHNOLOGIES IN THE PRESERVATION OF ENDANGERED PLANT AND ANIMAL SPECIES

SPONSORS: ZOOLOGICAL SOCIETY OF SAN DIEGO, CENTER FOR RESEARCH OF ENDANGERED WILDLIFE CINCINNATI ZOO AND BOTANICAL GARDEN, INC.

Conveners: Barbara S. Durrant, PhD, Head, Reproductive Physiology Division, Zoological Society of San Diego and San Diego State University
Valerie Pence, Center for Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden

8:00 – 10:00 General Symposium Royal Palm 5 & 6
(See abstracts page 6-A)

In vitro technologies developed for economically important species are now being used to preserve and propagate endangered plant and animal species. This session will discuss some of the problems facing endangered species and the ways in which in vitro methods are being applied to assist in the conservation of these taxa.

8:00 Introduction (B.S. Durrant)
8:15 JS-22 In Vitro Propagation for Ex Situ Preservation of Endangered Plant Species
Valerie C. Pence, PhD, *Plant Conservation Division, Center for Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden*
8:40 JS-23 In Vitro Techniques for the Propagation of Endangered Plants Endemic to Hawaii
Charles H. Lamoureax, PhD, *Director, Harold L. Lyon Arboretum, University of Hawaii, Manoa*
9:05 JS-24 The Role of the "Frozen Zoo" in Genetic Research for the Preservation of Endangered Species
Maryls Houck, *Cytogenetics Division, Center for Reproduction of Endangered Species, Zoological Society of San Diego*
9:30 JS-25 Development of In Vitro Oocyte Maturation and Cryopreservation Techniques for the Domestic Dog as a Model for Germplasm Rescue in Endangered Carnivores
Barbara S. Durrant, PhD, *Center For Reproduction of Endangered Species, Zoological Society of San Diego and San Diego State University*

Tuesday, June 13

MOLECULAR ASPECTS OF PLANT DISEASE

Moderator: David Songstad, PhD, Monsanto

8:00 – 10:00 — Plant Contributed Paper Session — Pacific 4 & 5

(See abstracts page 43-A to 45-A)

8:00 P-1020 Characterization of Differentially Expressed Extracellular Proteins After In Vitro Selection for Anthracnose Resistance in Grapevine
S. Jayasankar, *Univeristy of Florida, Z.J.Li and, D.J. Gray*

8:15 P-1021 A Study on the Expression of the Grapevine *Vitivirus A* Movement Protein Gene in Transgenic Grapevines
Lucia Martinelli, *Instituto Agrario Provincale, E. Candioli, F. Dalla Vecchia, A. Minatra, and N. Rascio*

8:30 P-1022 Engineering Disease Resistance in Wheat by Cloning Defense Genes
Ajith Anand, *Kansas State University, T. Zhou, R. D. Wamsley, V. Janakiraman, S. Prakash, W. Li, W. Chen, N. Sakthivel, B. Gill, G. Liang, J. Shah, H. N. Trick, and S. Muthukrishnan*

8:45 P-1023 Effect and Expression *hrpN* in Apple on Resistance to *Erwina amylovora*
Ewa Borejsza-Wysocka, *Cornell University, A. A. Kader, J.L. Norelli, D.W. Bauer, E. R. Garr, S. V. Beer, and H.S. Aldwinckle*

9:00 P-1024 Caspase Inhibitors Block Pathogen Proliferation and Symptoms of Compatible and Incompatible Plant-Pathogen Interactions
Craig Richael, *University of California, J.E. Lincoln, R. Bostock, D. Gilchrist*

9:15 P-1025 Transformation and Characterization of a Thaumatin like Antisense Gene of Barley in Wheat for Increased Fungal Resistance
Alessandro Pellegrineschi, *CIMMYT, M. M. Salgado, S. McLean, and D. Hoisington*

9:30 P-1026 Resistance of Attacin E Transgenic Lines of Royal Gala Apple to *Ervinia amylovra* (Fire Blight)
Herb S. Aldewinckle, *Cornell University, J.L. Norelli, E. Borejsza-Wysocka, J.-P. Reynoird, and M.V. Bhaskara Reddy*

INSECT CELL CULTURE APPLICATIONS

Moderator: Guido Caputo, Great Lakes Forestry Centre, Sault St. Marie, Ontario

8:00 – 10:00 — Invertebrate Contributed Paper Session — Pacific 6 & 7

(See abstracts page 38-A)

8:00 I-1009 In Vivo and In Vitro Effects of a Fat Body Extract on Spodoptera littoralis
Guy Smagghe, *Ghent University, M. Loeb, and L. Tirry*

8:20 I-1010 Immunohistochemical Localization of MDF1 (Midgut Cell Differentiation Factor 1) in Midgut Cells of Heliothis virescens
Shintaro Goto, *Kobe University, M. Takeda, R.S. Hakim, and M. J. Loeb*

8:40 I-1011 Establishment of a Novel Bombyx mori Cell Line That Reacts Hemolymph and Changes Cell Form
Shigeio Imanishi, *National Institute of Sericultural and Entomological Science, S. Tomita, M. Kiuchi, and M. Kamimura*

9:00 I-1013 Juvenile Hormones and Juvenile Hormone Mimics Inhibit ^{14}C-GlcNAc Uptake and Proliferation in an Indian Meal Moth Cell Line Derived from Wing Imaginal Discs
Herbert Oberlander, *U S Department of Agriculture, ARS, E. Leach, and E. Shaaya*

Tuesday, June 13

10:00 - 10:30 Coffee Break Grand Ballroom

10:00 - 15:00 Exhibits and Posters Grand Ballroom

DISTINGUISHED PLENARY SESSION

SPONSOR: CORNING SCIENCE PRODUCTS

Conveners: Sandra L. Schneider, DrPH, Research and Clinical Laboratory Systems
Hank Lane, Navicyte, Inc.

10:30 – 11:30 Distinguished Plenary Session Town & Country Room

Advances in Understanding the Molecular, Genetic and Protein Structure of Neurodegenerative Diseases

(See abstract page 1-A)

Introduction (S. L. Schneider and H. Lane)

PS-2 ***Stanley B. Prusiner, MD**, Director, Institute for Neurodegenerative Diseases, Professor Neurology and Biochemistry, University of California, San Francisco and 1997 Nobel Laureate in Physiology or Medicine "For His Discovery of Prions …"*

11:30 – 13:00 **Distinguished Plenary Reception** Grand Ballroom

SPONSOR: CORNING SCIENCE PRODUCTS

Tuesday, June 13
All Poster Authors will be present
12:00 – 12:30
(See list of posters on pages)

13:00 - 14:00 Poster Removal Grand Ballroom

CELL DEATH/CELL CYCLE CONTROL IN PLANTS

SPONSOR: DNAP PLANT TECHNOLOGIES, NOVARTIS

Conveners: Ebe Firoozabady, PhD, DNA Plant Tech Corporation
David Gilchrist, PhD, University of California, Davis

13:30 - 15:30 Plant Workshop Royal Palm 3 & 4

(See abstracts page 33-A)

Characterization of the cell cycle and programmed cell death is emerging as dual interests in plant biology. Understanding the relationship of the cell cycle through apoptosis, or programmed cell death, in animals has revealed a functionally connected set of regulatory signal pathways. "Kingdom-crossing" connections between the cell cycle and

programmed cell death in plants and animals are of fundamental interest. Many of the genes that regulate the cell cycle in plants have been cloned and appear to be functional analogous to those operating in other eukaryotic cells. Manipulation of the cell cycle holds promise to understand and genetically modify the development of plants, as well as the response of plants to a number of external stimuli, including disease. Programmed cell death in plants has been demonstrated to share a number of the morphological characteristics of apoptosis in animal systems. The characterization of programmed cell death in plants has focused on the induction or suppression of cell death in plant disease. This symposium will focus on the role of programmed cell death in both resistant and susceptible responses of plants to microbial pathogens, and the potential of reducing disease by genetic engineering of programmed cell death pathways.

13:30 Introduction (E. Firoozabady)

13:45 W-11 Causal Connections: Programmed Cell Death, the Cell Cycle and Plant Disease
David Gilchrist, PhD, *Associate Director CEPRAP, University of California, Davis*

14:15 W-12 Apoptosis-like Cell Death in a Plant Disease Response
Thomas J. Wolpert, PhD, *Dept. of Botany and Plant Pathology, Oregon State University, Inc.*

14:45 W-13 Maize D-type Cyclin Genes and Their Potential for Improving Transformation
Yumin Tao, PhD, *Traits And Technology Department, Pioneer Hi-Bred International, Inc.*

15:15 W-14 The Ins and Outs of the Plant Cell Cycle
Peter Casteels, PhD, *Department of Genetics, University of Gent, Belgium and Vlaams Instituut Voor Biotechnologie*

STEM CELL BIOTECHNOLOGY

SPONSOR: ADVANCED CELL TECHNOLOGY, INC

Convener: James Robl, PhD, Advanced Cell Technology Inc

13:30- 15:30 Vertebrate Symposium Royal Palm 5 & 6

Science in the new millennium brings with it the promise that aging and damaged organs will be replaced from spare parts "grown" in the laboratory. This rejuvenation technology may be in the form of matrix organs and/or the ability to repopulate malfunctioning organs with viable cells that express a specific cellular function. The promise of this technology is possible through achievements in the field of stem cell research. Methods have been devised to isolate, culture and maintain human embryonic and fetal stem cells in a primordial lineage or uncommitted state. These cells can then be induced to undergo organ specific differentiation. Studies have negated the long held belief that adult-derived stem cells have a limited differentiation repertoire. Populations of stem cells exist in some adult tissues that retain the potential to differentiate into cell types other than those of their origin. It has been demonstrated that stem cells taken from muscle can be induced to become blood cells, while stem cells from rat bone marrow can differentiate into either/or liver and neural cells. This SID will discuss the current state of stem cell research and the approaches to use their potential for tissue replacement and regeneration.

13:30 Introduction (A. Vidrich and J. Robl)

13:45 V-23 Hematopoetic Stem Cells and Neural Trans-differentiation
Irving Weissman, PhD, *Department of Pathology, Stanford University*

14:15 V-24 Potential Approaches For Myocardial Regeneration
Loren Field, PhD, *Krannert Institute of Cardiology, Indiana University School of Medicine*

14:45 V-25 Approaches for Production and Genetic Modification of Embryonic Stem Cells
Jose Cibelli, PhD, *Tissue Engineering & Transplant Medicine, Advanced Cell Technology, Inc.*

MOLECULAR BIOLOGY OF FISH CELLS IN VITRO

SPONSORS: AMERICAN TYPE CULTURE COLLECTION AND KENT SEA TECH CORPORATION

Convener: David Barnes, PhD, American Type Culture Collection

13:30 - 17:30 Joint Vertebrate/Invertebrate/Cellular Toxicology Symposium Pacific 4 & 5

(See abstracts page 7-A to 8-A)

The study of fish species represents not only a practical approach to improvement of food biotechnology, but also provides basic research models of development, toxicology, immunology, pathology and virology. In vitro approaches are finding an increasing role in fish cell biology and genetics. This symposium will examine some recently developed tools for cellular and molecular analyses of fish development and genetics, as well as applications of in vitro approaches to understanding fish diseases and improving food biotechnology.

13:30 Introduction (D. Barnes)

13:40 JS-26 Pantropic Retroviral Vectors For Gene Expression In Fish Cell Lines: Toward A Rational Approach to Genetic Engineering of Disease Resistance in Fish
Jane C. Burns, MD, *Department of Pediatrics, University California, San Diego*

14:05 JS-27 Fish Embryo Cell Cultures For Cell-Mediated Gene Transfer
Paul Collodi, PhD, *Purdue University*

14:30 JS-28 In Vitro Cultivation and Characterization of Viruses on Marine Organisms
Charles Buck, PhD, *Director of Virology, American Type Culture Collection*

14:55 JS-29 Algal Phycocyanins Promote Growth of Animal Cells in Culture
Kazuki Shinohara, PhD, *National Food Research Institute, Tsukuba*

15:20 – 15:35 Session Break

15:35 JS-30 FGF Inhibits Neurogenesis In Zebrafish Early Embryo Cells
David Barnes, PhD, *American Type Culture Collection*

16:00 JS-31 Prospective Acute Phase Proteins Expressed In Hepatocytes of Rainbow Trout
Christopher Bayne, PhD, *Department of Zoology, Oregon State University*

16:25 JS- 32 Establishment of An Abalone Digestive Gland Cell Line Secreting Various Glycosidases in Protein-free Culture
Sanetaka Shirahata, PhD, *School of Genetic Resources Technology, Kyushu University*

16:50 JS-33 The Manazar Project: A Low-Tech Approach To Maricultue On Desert Shorelines
Gordon Sato, PhD, *Ministry of Fisheries, Massawa*

IN VITRO MODELS FOR THE STUDY OF CANCER

Moderator: Eugene L. Elmore, PhD, University of California, Irvine

14:00 - 15:30 Vertebrate/Cellular Toxicology Contributed Paper Session Royal Palm 2

(See abstracts page 49-A to 50-A)

14:00 VT-1004 RNA Microarray Analysis of DFMO Induced Changes in Cancer-Related Gene Expression in Two Pre-cancerous Human Colon Polyp Cell Lines
Eugene L. Elmore, *University of California, Irvine, R.A. Lubet, J.L. Redpath, G. J. Kelloff, and V. E. Steele*

14:15	VT-1005	In Vitro Tissue Culture Models to Study the Interaction of Human Papillomavirus and Other Viruses of the Female Genitourinary System ***Craig Meyers,*** *The Pennsylvania State College of Medicine, S. Alam, S. Andreansky, R.J. Courtney, M. Mane and P.L.Hermonat*
14:30	VT-1006	Expression and Function of *Sonic hedgehog* and *Patched* Genes in Oral Epithelial Cells and Oral Squamous Cell Carcinoma Cell Lines ***Eiji Michimukai,*** *Hiroshima University Faculty of Dentistry, Y. Tanaka, N. Kitamura, S. Toratani, and T. Okamoto*
14:45	VT-1007	Measurement of the Barrier Function of Cell Layers During a Metastatic Cell Challenge ***Charles R. Keese,*** *Applied Biophysics, Inc., K. Bhawe, and I. Giaever*
15:00	VT-1001	Signal Transduction in Lymphocyte Locomotion: Microgravity Induced Lesions ***Alamelu Sundaresan,*** *Wyle Laboratories, D. Risin, and N.R. Pellis*
15:15	VT-1002	Transplantation of Mouse Pancreatic Islets Cultured in Microgravity-Stimulating Bioreactors ***Lynne P. Rutsky,*** *University of Texas Health Science Center Medical School, M. Kloc, S. Bilinski, S. Stepkowski, T. Phan, H. Zhang, and S. Katz*

NEURAL STEM CELL DEVELOPMENT
SPONSORS: LIFE TECHNOLOGIES, INC.,
DEFENSE ADVANCED RESEARCH PROJECTS AGENCY (DARPA)

Convener: Theo D. Palmer, PhD, Laboratory of Genetics, Salk Institute for Biological Studies

15:30 - 17:30 Joint Vertebrate/Invertebrate/Cellular Toxicology Symposium Royal Palm 1
(See abstracts page 9-A)

Neural precursors in the adult mammal take many forms. The most immature forms, the neural stem cell, retain considerable phenotypic plasticity and can generate a variety of glial and neuronal cell types. In vivo, an abundant population of neural stem cells resides within a residual ventricle-associated germinal matrix. Progeny from these cells contribute new neurons to the olfactory bulb, prefrontal cortex and hippocampus throughout adult life. The unexpected abundance of new neurons generated over an entire life span leads to the interesting concept that the "cues" necessary for repair and regeneration may play an active role in the adult brain. Knowledge of how these cues direct neural precursor fate and phenotype would yield insight into how to repair injury or degeneration in the post-developmental brain. In vitro systems have provided powerful tools for unraveling the signaling behind stem cell fate and led to several surprising insights. Not only can primary stem-like cells be propagated in culture, but many of these early "neural" precursors appear able to adopt phenotypes well beyond that expected for their site of origin. On going *in vitro* and via studies are exploring stem cell plasticity in programming cells for functional restoration within and, perhaps, beyond the central nervous system.

15:30		Introduction (T. Palmer)
15:45	JS-34	Reconstructing the Brain: From Stem Cells to Synapses ***Ronald D. G. Mckay, PhD,*** *Laboratory Chief, National Institute of Neurological Disorders and Strokes (NINDS), Bethesda*
16:15	JS-35	Neural Stem and Progenitor Cells in the Mammalian Brain ***Derek van der Kooy, PhD,*** *Department of Anatomy & Cell Biology, University of Toronto*
16:45	JS-36	Stem Cells and Neurogenesis: Lessons from the Adult Hippocampus ***Theo D. Palmer, PhD,*** *Laboratory of Genetics, Salk Institute for Biological Studies*

GENES THAT CONTROL PLANT DEVELOPMENT AND THEIR POTENTIAL USE IN TISSUE CULTURE AND TRANSFORMATION

SPONSOR: PIONEER HI-BRED, INTERNATIONAL.

Convener: Bill Gordon-Kamm, PhD, Pioneer Hi-Bred International, Inc.

15:30 - 17:30 | Plant Workshop | Royal Palm 3 & 4

(See abstracts page 34-A)

Traditionally, researchers in plant tissue culture and transformation investigate how altering culture conditions will influence in vitro growth rate and morphogenesis. This session will provide preliminary information on these genetic switches: that may be triggered leading to a given tissue culture response; how these genes might be used in transgenics to alter in vitro growth; and basic information on genes involved in embryo or meristem formation. The goal of this symposium is to convey the power of these developmental genes, in terms of their use as probes and in manipulating plant growth. The genes that govern embryo development and meristem formation have particular relevance to researchers in plant tissue culture and transformation. Recently, a number of critical embryo or meristem-specific genes have been discovered. The molecular genetics of embryogeny as well as meristem formation and maintenance will provide some examples of how these genes can be used to understand and manipulate the tissue culture process.

15:30 Introduction (B. Gordon-Kamm)

15:45 W-15 Use of Maize LEC1 to Improve Transformation
Keith Lowe, PhD, *Pioneer Hi-Bred International, Inc.*

16:15 W-16 The Response of Maize Tissue Cultures to Different In Vitro Conditions; Characterized Using Embryo and Meristem Specific Probes
Peggy Lemaux, PhD, *University of California, Berkeley*

16:45 W-17 Knotted-1 and Its Influence on Meristems and Senescence
Sarah Hake, PhD, *US Department of Agriculture, ARS, Plant Gene Expression Center*

17:00 W-18 Genes that Control and Coordinate Embryogenesis in Higher Plants
John Harada, PhD, *University of California, Davis*

WEDNESDAY, JUNE 14

7:00 - 18:00 Registration Grand Foyer

MOLECULAR FARMING - PLANT BIOTECHNOLOGY'S NEXT BIG WAVE

SPONSOR: PRODIGENE

Convener: Mike Horn, PhD, ProdiGene

8:00 – 10:00 Plant Symposium Royal Palm 3 & 4

(See abstracts page 20-A)

Plants are being recognized as the perfect bioreactors for producing proteins of industrial and pharmaceutical uses. Cost of protein production using plants can be 100-200-fold less than conventional bioreactors. Moreover, the limiting factor is available acreage rather than bioreactor volumes. Several products are already on the market and this field is poised for incredible growth. This session will have a cross-section of speakers covering at the product areas: edible vaccines, monoclonal antibodies and industrial enzymes. This symposium will address the potential market size, regulatory hurdles, technological challenges and advantages of using plant systems.

8:00 Introduction – an Overview of the Molecular Farming Industry
Michael Horn, PhD, *ProdiGene*

8:15 P-21 Plantibodies for Reproductive Health: Herpes Therapy and Prevention
Kristen Briggs, PhD, *Epicyte Pharmaceuticals*

8:40 P-22 Oral Delivery of Subunit Vaccines Using Corn
Stephen Streatfield, PhD, *Prodigene*

9:05 P-23 Monoclonal Antibodies from Corn Seed
Julio A. Baez, PhD, *Integrated Protein Technologies*

9:30 P-24 Use of Plant Roots for Biomedical Manufacturing
Ilya Raskin, PhD, *Biotech Center, Rutgers University*

PROTEINASES AND INHIBITORS IN ANGIOGENESIS

Convener: James J. Quigley, PhD, Dept. of Vascular Biology, The Scripps Research Center

8:00 – 10:00 Vertebrate Symposium Royal Palm 2

(See abstracts page 28-A to 29-A)

The induction and formation of new blood vessels within normal and pathologic tissue requires both subtle and extensive proteolytic remodeling of tissue extracellular matrix. The nature of the induced proteinase systems, to include: mechanism of action, control by specific inhibitors and the identity of their substrates are still unresolved. In this symposium, some of these unresolved questions will be discussed and experiments addressing the molecular, cellular and biochemical events that occur during angiogenic remodeling will be presented. A number of informative via and *in vitro* angiogenic model systems will be presented.

8:00 Introduction (J. Quigley)

8:15 V-26 Involvement of MMP Proteinase Systems and Specific Collagen Cleavage in a Quantitative Angiogenesis Model
Marco Seandel, PhD, *State University of New York at Stony Brook*

8:45 V-27 MMP-9 Binds to a Ligand Induced Cryptic Site Within b1 Intergrin: Role in Angiogenesis and Tumor Growth
Peter Brooks, PhD, *Department of Biochemistry and Molecular Biology, Norris Cancer Center, University Southern California, Los Angeles*

9:15 V-28 Inhibition of Angiogenesis by Plasminogen Activator Inhibitor
Daniel A. Lawrence, PhD, *American Red Cross*

SOMATIC EMBRYOGENESIS

Moderator: Wayne Parrot, PhD, University of Georgia

8:00 - 10:00 Plant Contributed Paper Session Royal Palm 5 & 6
(See abstracts page 45-A to 46-A, 48-A)

8:00 P-1027 Somatic Embryogenesis from Isolated Tissues of Mature Zygotic *Pinus palustris*
Alex M. Diner, *USDA Forest Service, SFRD*

8:15 P-1028 Walnut Trees That Flower In Vitro: Micropropagation, Somatic Embryogenesis, Plant Regeneration and Genetic Transformation
Christian Breton, *INRA-Orleans, D. Cornu, P. Capelli, D. Chriqui, L. Chretien, E. Germain, and C. Jay-Allemand*

8:30 P-1029 Increasing the Yield of Maturing Loblolly Pine Somatic Embryos Through Simultaneous Optimization of Cell Density and ABA Concentration
Teresa Vales, *Institute of Paper Science and Technology, and G.F. Peter*

8:45 P-1030 Direct Somatic Embryogenesis Through Pseudo-Bulbet Thin Cell Layer of *Lilium longiflorum*
Duong Tan Nhut, *Kagawa University, B.V. Le, S. Kukai, M. Tanaka, K. Tran Thanh Van*

9:00 P-1031 Image Analysis: A Practical Approach for Evaluation of Soybean (*Glycine max*, L. Merrill) Somatic Embryo Growth
Marco T. Buenrostro-Nava, *Ohio State University, H.M. Frantz, P.P. Ling, and J.J. Finer*

9:15 P-1032 Isolation of Genes Expressed During Somatic Embryogenesis on Orchardgrass
Krassimira S. Alexandrova, *University of Tennessee, and B.V. Conger*

9:30 P-1038 Purine and Pyrimidine Metabolism During the Partial Drying Treatment and Germination of White Spruce (*Picea glauca)* Somatic Embryos
Claudio Stasolla, *The University of Calgary, N. Loukanina, I. Ashihara, E.C. Yeung, and T.A. Thorpe*

10:00 - 10:30 Coffee Break Grand Foyer

Wednesday, June 14

INSECT MIDGUT

SPONSOR: INVITROGEN

CO-SPONSOR: NOVARTIS AGRIBUSINESS BIOTECHNOLGY RESEARCH, INC.

Convener: Marcia J. Loeb, PhD, Insect Reproduction Laboratory, US Department of Agriculture

8:00 – 12:30 Invertebrate Symposium Royal Palm 1

(See abstracts page 14-A to 15-A)

The midgut is the largest internal organ in insect larvae; through it passes all of the food as well as pathogens or toxic materials ingested. Substances, such as *Bacillus thuringiensis* (Bt) toxin destroy midgut cells, leading to death by septicemia; viruses enter, often multiply in midgut cells before passing to other organs in the body. Injured midgut has the capacity to repair itself, and thus reverse toxin or pathogen damage. The midgut depends on undifferentiated stem cells for this effect. The midgut has the intrinsic capacity to: direct stem cells to multiply; differentiate to one of several mature forms; or induce decreases in cell number in case of starvation, by means of cytokines secreted by the midgut cells themselves. Therefore, the insect midgut is of interest to developmental biologists. This system provides a simple non-toxic stem cell system and working model.

8:00 Introduction (M. Loeb)

8:10 I-10 Capabilities of Stem Cells from the Lepidopteran Midgut
Raziel S. Hakim, PhD, *Department of Anatomy, Howard University College of Medicine*

8:45 I-11 Cockroach Midgut Peptides and Monoamines that Regulate Cell Proliferation, Differentiation, and Death
Makio Takeda, PhD, *Faculty of Agriculture, Kobe University*

9:20 I-12 Control of Life and Death, with Indications for Midgut Repair in Cultured Midgut Cells from the Lepidopteran, *Heliothis virescens*
Marcia J. Loeb, PhD, *Insect Reproduction Laboratory, US Department of Agriculture*

9:55 I-13 Transport Processes in the Insect Midgut
Sarjeet S. Gill, PhD, *Department of Neuroscience, University of California, Riverside*

10:30 – 11:00 Invertebrate Session Coffee Break Foyer

11:00 I-14 Primary and Established Midgut Cultures from *Pseudaletia unipuncta* and *Trichoplusia ni* Larvae for Baculovirus Studies
Robert Granados, PhD, *Boyce Thompson Institute*

11:35 I-15 In Vitro Hemocyte and Tissue Assays for Assessing Baculovirus Interactions with Their Host Insects
Jan O. Washburn, PhD, *Department of Plant and Microbial Biology, University of California, Berkeley*

12:10 Discussion

Wednesday, June 14

NEW TECHNOLOGIES FOR ADVANCING DRUG DEVELOPMENT & DISCOVERY

SPONSOR: TREGA BIOSCIENCES, INC.

Convener: Patrick Sinko, PhD, Trega Biosciences, Inc.

10:30 - 12:30 — Cellular Toxicology Symposium — Royal Palm 2

(See abstracts page 22-A to 23-A)

The importance of in vitro biology in the selection of drug candidates is growing rapidly. The need for faster and more efficient methods and screening processes are the direct consequences of the changes and advancements in the drug discovery paradigm. With the addition of high-throughput screening and the creation of computationally diverse compound libraries through combinatorial chemistry, a very large number of prospective drug candidates are being identified. The process of converting these "hits" to "leads" is of enormous importance and is the measure of success of the new drug discovery paradigm. This session will concentrate on highlighting the changes in the early stages of drug discovery from drug design and synthesis to high-throughput in *silico* and in vitro biological (pharmacological and pharmacokinetic) screening.

10:30 Introduction (P. Sinko)

10:45 T-4 New Strategies for the Acceleration of the Drug Discovery Process
David C. Myles, PhD, *Organic and Medicinal Chemistry, Chiron Corporation*

11:15 T-5 Integrating Biology with New Drug Discovery Paradigm
Kia Motesharei, PhD, *New Technologies, Trega Biosciences, Inc.*

11:45 T-6 Chemistry-Space Concepts and Their Use for Accelerated Drug Discovery
Robert S. Pearlman, PhD, *Laboratory for Molecular Graphics and Theoretical Modeling, College of Pharmacy, University of Texas, Austin*

ALTERNATIVE MARKERS FOR PLANT TRANSFORMATION

SPONSOR: PIONEER HI-BRED, INTERNATIONAL

Conveners: Heidi Kaeppler, PhD, University of Wisconsin, Madison
Matthew Bailey, PhD, Pioneer Hi-Bred International

10:30 - 12:30 — Plant Workshop — Royal Palm 3 & 4

(See abstracts page 34-A to 35-A)

Herbicide and antibiotic resistance genes have been the most widely utilized selectable markers for genetic engineering of plants. Despite the successful application of these genes, new selectable markers need to be developed to increase the efficiency and flexibility of plant transformation. In addition, public concern about the presence of certain selectable marker genes in plants used for food and those released into the environment has encouraged further research in designing alternative markers and selection systems. Speakers in this workshop will describe several new selectable markers and selection systems recently developed for efficient plant transformation. Technical details, helpful hints, advantages and disadvantages for each of the systems will be discussed.

10:30 Introduction (H. Kaeppler and M. Bailey)

10:45 W-19 The Use of Phosphomannose Isomerase (PMI) as an Efficient Selectable Marker for Monocot and Dicot Transformation
Janet Reed, PhD, *Novartis*

11:10 W-20 Plant Selection Principle Based on Xylose Isomerase
Anna Haldrup, PhD, *Royal Veterinary and Agricultural University*

11:35 W-21 MAT (Multi-Auto-Transformation) Vector: "The Oncogenes of Agrobacterium are Used as Positive Markers for Regeneration and Selection of Marker-Free Transgenic Plants"
Hiroyasu Ebinuma, PhD, *Nippon Paper Industries*

12:00 W-22 Seeing the Light: Utilization of GFP as a Visual Screenable Marker for Plant Transformation
Heidi Kaeppler, PhD, *University of Wisconsin, Madison*

CHARACTERIZATION OF ANIMAL CELLS: VIRAL CONTAMINATION AND AUTHENTICITY

SPONSOR: JRH BIOSCIENCES

CO-SPONSORS: ASHBY SCIENTIFIC LTD., EUROPEAN TISSUE CULTURE SOCIETY, KLUWER ACADEMIC PUBLISHERS, LABORATORY OF THE GOVERNMENT CHEMIST, TRIPLE RED, SIGMA

Conveners: R. Ian Freshney, MD, University of Glasgow
Glyn N. Stacey, PhD, Division of Virology, National Institute for Biological Standards and Control

12:30 - 14:30 — Vertebrate Symposium — Royal Palm 2

(See abstracts page 29-A to 30-A)

The expanding use of cell culture means that there are many workers new to this technique who may not have the opportunity to gain a knowledge of some the fundamental issues in cell culture. The goal of this symposium is to raise awareness on two critical cell line issues: cell line authenticity and virus contamination. Techniques are now available that make cell authentication a routine procedure. These state of the art techniques and new data on cross contamination of cell lines will be presented. Viral contamination of cell lines can significantly influence or invalidate scientific data and cell line products. Such contamination may also represent a health risk to laboratory workers. The scientific basis for risk assessment of cell cultures will be discussed. Virus testing regimes applied to cell lines used in manufacture of biological products will be discussed, as well as new protocols for the elimination of viruses from serum. The approaches to address the problems of cell authentication and potential viral contamination of tissue culture reagents will be discussed by a panel of experts in the field of in vitro biology.

12:30 Introduction (R.I. Freshney and G.N. Stacey)

12:45 V-29 Adventitious Agents in Source Materials: Cells and Growth Media
Glyn N. Stacey, PhD, *Division of Virology, National Institute for Biological Standards and Control*

13:05 V-30 Gamma-irradiated Serum: A Validated Method to Inactivate Viral Contaminants and Conserve Product Integrity
Richard Festen, PhD, *JRH Biosciences*

13:25 V-31 DNA Profiling and Cross-Contamination of Human Cultures Lines
John Masters, PhD, *University College London*

13:45 V-32 Cell Line Authentication: Cross-Contaminations and Misidentifications
Hans G. Drexler, MA, PhD, *Department of Human and Animal Cell Cultures, DSMZ, German Collection of Microorganisms and Cell Cultures*

14:05: V-33 A Profile of the ATCC Human Cell Line Collection
A. Scott Dirkin, MD, *American Type Culture Collection*

FACTORS AFFECTING CELLULAR GROWTH AND DIFFERENTIATION (I)

Moderator: Mutsumi Takagi, PhD, Osaka Univeristy

13:00 –14:00 — Vertebrate/Toxicology Contributed Paper Session — Royal Palm 1

(See abstracts 50-A to 51-A)

13:00 VT-1011 Expansion of Murine Hematopoietic Progenitor Cells Population with Spatial Development of Stromal Cells in Porous Carriers without Cytokine Addition
Mutsumi Takagi, *Osaka University, T. Sasaki and T. Yoshida*

13:15 VT-1009 Involvement of Muscarinic Acetylcholine Receptor (mAchR) in Cytotoxicity of an Organophosphate Anticholinesterase, Diisopropylfluorophosphate (DFP)
Cheng J. Cao, *US Army Edgewood Chemical Biological Center, R.J. Mioduszewski, D.E. Menking, A.T. Eldefrawi, and J.J. Valdes*

13:30 VT-1010 Regulation of Differentiation Balance Between Osteogenesis and Adipogenesis by Gene Transfection
Masayasu Mie, *Tokyo Institute of Technology, H. Ohgushi, Y. Yanagida, T. Haruyama, E. Kobatake, and M. Aizawa*

FACTORS AFFECTING CELLULAR GROWTH AND DIFFERENTIATION (II)

Moderator: Yoshinobu Matsuo, Hayashibara Biochemical Labs, Inc.

13:00 –14:00 Vertebrate/Toxicology Contributed Paper Session (See abstracts page 51-A to 52-A) Royal Palm 2

13:00 VT-1012 Growth Arrest Related eti-1 Gene (Epthelial Topoinhibition Inducible) with 6 RCC1 Repeats Induces Apoptosis
Tohru Masui, *National Institute of Health Sciences, Tokyo, S. Iwashita Y Takada, M. Hayashi, and H. Mizusawa*

13:15 VT-1013 Disorder of Intracellular Localization of S100C in the Process of Immortalization of Normal Human Fibroblasts
Masakiyo Sakaguchi, *Okayama University Medical School, and M. Namba*

13:30 VT-1014 Inflammatory Activation of Neutrophils Triggered by a Novel Class of Peptides
Hidehito Mukai, *Japan Tobacco, Inc., Y. Hokari, T. Seki, H. Nakano, T. Takao, Y. Shimonishi, E. Munekata, and Y. Nishi*

13:45 VT-1015 Cross-contaminations of Human Leukemia-Lymphoma Cell Lines
Yoshinobu Matsuo, *Hayashibara Biochemical Labs, Inc., C. Nishizaki, W.G. Dirks, S. Habig, and H.G. Drexler*

THE REVOLUTION IN MOLECULAR AND CELLULAR BIOLOGY PRESENTS UNIQUE CHALLENGES AND OPPORTUNITIES FOR HIGH THROUGHPUT SCREENING APPLICATION

SPONSOR: ELI LILLY and COMPANY

Convener: Dennis Laska, Eli Lilly and Company

14:30 - 16:30 Cellular Toxicology Symposium (See abstracts page 23A) Royal Palm 1 & 2

Identification of new and unique drug targets and cellular markers, as well as the advances in high throughput screening technology (HTS), have created an explosion of promising new drug candidates in the Pharmaceutical Industry. As the numbers of lead candidates multiplies, scientists in Pharmaceutical Development are challenged with the task of sorting the leads to identify the safest and most effective compounds. To best handle the increased throughput, scientists in Toxicology and Drug Disposition (Tox/ADME) must be innovative in their application of molecular biology, genomics, proteomics, computational chemistry, and HTS as they validate cell-based, surrogate models to aid in lead candidate selection. The presenters for this symposium will discuss the development of unique target cells and provide insight on their application in HTS.

14:30 Introduction (D. Laska)

14:45 T-7 Developing Target Cells, Representative of Differentiated Cell Types, Using Targeted Oncogenesis in Transgenic Mice
Pamela L. Mellon, PhD, *Center For The Study of Reproductive Biology and Disease, University of California School of Medicine, San Diego*

15:15 T-8 Genetic Programs of Hematopoietic Stem Cells and Microenvironments
Ihor Lemischka, PhD, *Department of Molecular Biology, Princeton University*

15:45 T-9 Challenges and Solutions to Cell-based High-throughput Screening and Lead Optimization
Thomas H. Large, PhD, *Sphinx Pharmaceuticals, Division of Eli Lilly and Company*

BEYOND SCIENCE: IMPACT OF AGRICULTURE BIOTECHNOLOGY ON PRODUCTION, FOOD AND SOCIETY

SPONSORS: UST, INC., PIONEER HI-BRED INTERNATIONAL, INC.

Conveners: Dwight Tomes, PhD, Pioneer Hi-Bred International, Inc.
Peggy Lemaux, PhD, University of California, Berkeley

14:30 - 16:30 Plant Symposium (See abstracts page 21-A) Royal Palm 3 & 4

Genetically Modified Foods (GMOs) have received a great deal of attention in the past year. This is particularly true in Europe where recent food quality scandals over "mad cow disease" and dioxin contamination of feed and food have eroded public confidence in government policymakers. Several politically motivated action groups have taken advantage of these food quality issues to attack GMOs. These attacks have often been without any pretense of scientific fact, and/or to prevent adoption of GMOs with improved input trait characteristics. An example of this is the selected and biased information on. Bt containing corn, which reduces environmental damage from more dangerous pesticides and herbicides. This symposium will present a balanced view of GMO issues in the context of scientific data, changes in worldwide agricultural economics, the role of technically trained scientists addressing these issues, and how government policy can promote credibility in this area.

14:30 Introduction (D. Tomes and P. Lemaux)

14:45 P-25 Knowledge Generation and Transfer: The Case of Biotechnology
Gordon C. Rausser, PhD, *University of California, Berkeley*

15:10 P-26 Biotechnology in the Developing World: Challenges and Opportunities
C.S. Prakash, PhD, *Tuskegee University*

15:35 P-27 Public Perceptions of Agriculture Biotechnology: Implications for Biologists
Tom Hoban, PhD, *North Carolina State*

16:00 P- 28 FDA Policy on Bioengineered Plant Foods
Janet McDonald, PhD, *University of California, Berkeley*

17:00 - 18:00 SIVB Business Meeting (All Members are Urged to Attend) Royal Palm 5 & 6

19:00 – 20:00 **Reception/Silent Auction** Grand Ballroom Foyer

20:00 – 22:00 **Millennium Banquet** Town and Country Room
Seating is limited. Admittance to Banquet by Advance Ticket Holders only.

THURSDAY, JUNE 15

7:00 - 12:00 Registration Town and Country Foyer

BT-TRANSGENIC CROPS: EFFICACY, ENVIRONMENTAL EFFECTS, AND RESISTANCE MANAGEMENT

SPONSOR: UNIVERSITY OF CALIFORNIA BIOTECHNOLOGY RESEARCH AND EDUCATION

Conveners: Brian Federici, PhD, Department of Entomology and Graduate Programs in Genetics and Microbiology, University of California, Riverside
Neal Stewart, PhD, Department of Biology, University of North Carolina-Greensboro

8:00 - 12:30 Joint Invertebrate/Plant Symposium California
(See abstracts page 9-A to 10-A)

Transgenes encoding insecticidal proteins from *Bacillus thuringiensis* (Bt) are being used extensively and effectively as a crop plant and insect management tool. In 2000, over 25% of the cotton and corn crop in the USA will use Bt proteins to control lepidopteran larvae infestation. The health benefits of these crops are significant reductions in chemical insecticide applications and lower levels of mycotoxins in the human food chain. Despite these benefits, concerns have been raised about the safety of Bt-crops and their possible negative effects on agricultural ecosystems. The purpose of this symposium is to treat these topics in an objective manner by reviewing the relevant data available in scientific studies. The presentations will cover a range of topics, to include: the mode of action and toxicology of Bt endotoxins; synthesis of these endotoxins in plants; endotoxin safety and effects on non-target organisms; and resistance management strategies. The origins of Bt-crops will be reviewed, as well as the future of insecticidal transgenic plant technology. In this symposium, insect and plant-oriented speakers will present scientific on a new technology that promises long-term benefits for human health and the environment, with no demonstrated significant risks.

8:00 Introduction (B. Federici and N. Stewart)

8:15 JS-37 The Structures of Cytoloytic Mechanism of *Bacillus thuringiensis* Insecticidal Toxins
David J. Ellar, PhD, *Department of Biochemistry, Cambridge University, Cambridge*

8:45 JS-38 Expression of Bt Toxin Genes in Plants: Implications for Transgene Design
Scott Diehn, PhD, *MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing*

9:15 JS-39 The Diversity of Bt-Transgenic Crops
William J. Moar, PhD, *Department of Entomology, Auburn University, Alabama*

9:45 JS-40 Issues Affecting Corn Growers' Decisions to Plant (or Not to Plant) Bt Corn
Kevin L. Steffey, PhD, *Department of Crop Sciences, University of Illinois, Champaign*

10:15 – 10:45 Coffee Break California Foyer

10:45 JS-41 Effects of Bt-transgenic Crops on Nontarget Organisms
Angelika Hilbeck, PhD, *Geobotanical Institute, Swiss Federal Research Station, Department of Agronomy*

11:15 JS-42 Bt Resistance: Got Refuge?
Bruce E. Tabashnik, PhD, *Department of Entomology, University of Arizona, Tucson*

11:45 JS-43 New Insecticidal Proteins for Use in Transgenic Crops
Brian A. Federici, PhD, *Department of Entomology and Graduate Program in Genetics, University of California, Riverside*

12:15 Discussion

Education Posters

SUNDAY, JUNE 11	MONDAY, JUNE 12	TUESDAY, JUNE 13
10:00-21:00	10:00-21:00	10:00-13:00

Posters mounted Saturday, June 10, 15:00 – 17:00 hrs.
Posters must be removed from Exhibit Hall by 14:00, June 13.
Authors will be present at their posters the following days and times:

SATURDAY, JUNE 10	SUNDAY, JUNE 11	MONDAY, JUNE 12	TUESDAY, JUNE 13
All Authors Present	Even Authors Present	Odd Authors Present	All Authors Present
19:30 – 20:30	13:00-13:30	13:00-13:30	12:00-12:30

METHODS FOR IN VITRO EDUCATION

E-2000 Training for the Future: Cell Culture and Biosafety Workshops
M. L. Smith, *University of California, San Diego, and Brenda J. Wong*

E-2001 Affordable Plant Tissue Culture for the Classroom
C. Stiff, *Kitchen Culture Kits, Inc., J. Clancy, A. Evancoe, and C. Fiegel*

E-2002 DNA Isolation - A Comparison of Two Methods for the Secondary School Science Classroom
P. Bossert, *Northport High School, A. Kepping, and J. Hsieh*

E-2003 LIGASE Science Education Program: Teacher Training and In-house Research Programs
Z. Zachar, *State University of New York at Stony Brook*

STUDENT REPORTS

E-2004 Investigation of Evolutionary Relationships Among Mammals Using the Polymerase Chain Reaction
Y. Han, P. Frake, and L. Paluska, *Northport High School*

E-2005 Chemically Modified Tetracycline 300 Inhibition of PMN Lysate Mediated Degradation of Alpha 1-Protease Inhibitor (alpha1-PI)
L. Paluska, *Northport High School*

E-2006 The Effect Gastrointestinal pH on Chemically Modified Tetracyclines, An In Vitro Analysis of Potential Methods of Drug Delivery.
P.C. Frake, *Northport High School, E.Roemer and S. Simon*

E-2007 Cloning and Analysis of the Heart-Inducing Tbx5 Transcription Factor Gene from the *Xenopus tropicalis* Genome
Y. Han, *Northport High School*

E-2008 Propagation of Daylily by Conventional and Non-conventional Methods.
T. Swann, *Houston County High School, J.Carter and S.Dhir*

E-2009 Introduction of Vaccine Genes in Tomato.
M. Shah, *Warner Robins High School and S.K. Dhir*

Education and Invertebrate Posters

EDUCATION SILENT ABSTRACT

E-2010 Initial Assessment of the Effects of Battery Dumping in the Tennessee River; Results from Lead and Mercury Testing in River Invertebrates
T. Harris, *Giles County High School*

INVERTEBRATE POSTER

I-2000 Down-Regulation of the Polyhedrin Gene by the Replication of an NPV and a GV in the Same Cell
G.F. Caputo, *Great Lakes Forestry Centre, S. S.Sohi, and B. M. Arif*

Plant Posters

SUNDAY, JUNE 11	MONDAY, JUNE 12	TUESDAY, JUNE 13
10:00-21:00	10:00-21:00	10:00-13:00

Posters mounted Saturday, June 10, 15:00 – 17:00 hrs.
Posters must be removed from Exhibit Hall by 14:00, June 13.
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All Authors Present	Even Authors Present	Odd Authors Present	All Authors Present
19:30 – 20:30	13:00-13:30	13:00-13:30	12:00-12:30

DISEASE RESISTANCE

P-2000 Programmed Cell Death in Fungi: Heterokaryon Incompatibility Involves Nuclear DNA Degradation
S.M. Marek, *University of California, Davis, J. Wu, N.L. Glass, D.G. Gilchrist, R.M. Bostock*

P-2001 Role of Retinoblastoma-Related Protein in Programmed Cell Death in Diseased Plants
P.J. Hoegger, *CEPRAP, University of California, Davis, J.M. Li, J.E. Lincoln, and D.G. Gilchrist*

P-2002 Insect Resistant Tropical Maize Developed Through the Introduction of a Fully Modified *Bacillus thuringiensis cry* IB Gene
N. Bohorova, *CIMMYT, Int., R. Frutos, M. Royer, N. Lecointe, P. Estañol , M. Pacheco, S. McLean, and D. Hoisington*

P-2003 Development and Field-Testing of Walnut Trees Expressing the cryIA(c) Gene for Lepidopteran Insect Resistance
C.A. Leslie *, University of California, G.H. McGranahan, S.L. Uratsu, J.S. Tebbets, P.V. Vail, and A.M. Dandekar*

P-2004 Transformation of Leaf Rust Susceptible Lines of Maize and Wheat with the Maize Rp1-D Rust Resistance Gene
M. Steinau, *Kansas State University, S.H. Hulbert, and H.N. Trick*

P-2005 Introduction of *cryIAc* and *bar* Genes into Kenaf (*Hibiscus cannabinus* L.)
M.M. Young, *Missippi State University, and N.A. Reichert*

P-2006 Functional Screening and Characterization of Tomato Genes That Block PCD Induced by TA Toxin Using the Agrobacterium rhizogenes Hairy Root Transformation System
Z.Q. Pan, *University of California, Davis, X.H. Fan, R.J. Haworth, J.E. Lincoln, R.M. Bostock, and D.G. Gilchrist*

P-2007 Differentially expressed genes in wounded stem tissue of chestnut in vitro shoots
R. Schafleitner, *Austrian Research Centers, and E. Wilhelm*

P-2008 Expression of a Caspase Inhibitor Protein in Tomato Plants Results in Decreased Disease
J. E. Lincoln, *University of California, Davis, C. Richael, J. Li, K. Smith, X. Fan, I. Crossley, R. M. Bostock, and D. G. Gilchrist*

Plant Posters

STRESS RESISTANCE

P-2009 Engineering Wheat for Improved Resistance to Water Deficit Stress
T. Abebe, *Oklahoma State University, A.C. Guenzi and B.C. Martin*

P-2010 Molecular and Biochemical Analysis of Selected Rice Lines Created by Desiccation Treatment of Callus Tissues
P. T. Dinh, *Institute of Biotechnology, L.T. Binh, L. T. Muoi, and M. Engin*

P-2011 NaCl Stress Induces an Increase in Abscisic Acid Levels in Cotton Callus Tissue
D.R. Gossett, *Louisiana State University-Shreveport, J. Carmody, S.W. Banks, and M.C. Lucas*

P-2012 Isolation and Characterization of Serine and Proline Rich Protein Encoding Gene from *Porteresia coarctata* T. Under Salt Stress Conditions
S. G. C. Babu, *CNRS, and V. D. Reddy*

WOODY SPECIES

P-2014 Comparative Studies of Somatic Embryogenesis in *Quercus rubra* and *Juglans nigra*
M.J. Bosela, *USDA Forest Service, and C.H. Michler*

P-2016 Purine and Pyrimidine Biosynthesis During White Spruce (*Picea glauca*) Somatic Embryo Maturation
C. Stasolla, *University of Calgary, N. Loukanina, I. Ashihara, T.A. Thorpe*

EDIBLE VACCINES

P-2018 Development of Transgenic Plants as Source of Edible Vaccine For Rinderpest Disease
A. Khandelwal, *Indian Institute of Science, N. Geetha, K. J. M. Vally, M. S. Shaila, and G. Lakshmi Sita*

P-2019 Transgenic Banana Containing the Hepatitis B Surface Antigen
J.M. Van Eck, *The Boyce Thompson Institute for Plant Research, S.M. Abend, X. Sawycky, H.S. Mason, and C.J. Arntzen*

P-2020 Production of Recombinant Rotavirus VP6 from a Suspension Culture of Transgenic Tomato (*Lycopersicon esculentum* Mill.) Cells
I. S. Chung, *Kyung Hee University, C.H. Kim, S.H. Hong, J.H. Park, J. J. Lee*

SECONDARY PRODUCTS

P-2021 Production of Catalpol in Hairy Root Cultures of Chinese Foxglove (*Rehmannia glutinosa*)
S.J.Hwang, *Dongshin University*

P-2022 Terpenoid Biosynthesis via a Non Mevalonic Acid Pathway in Transformed Roots of *Artemisia annua* L.: Cloning and Expression of DXS and DXR.
F.F. Souret, *Worcester Polytechnic Institute, K.K. Wobbe, and P.J. Weathers*

P-2023 Is an Ascorbate Peroxidase Involved in Degradation of Artemisinin in Hairy Roots?
T. Iskra, *Worcester Polytechnic Institute, K.K. Wobbe, and P.J. Weathers*

P-2024 Synthesis and Accumulation of Lipid Compounds in In Vitro Cultures of Cynara cardunculus L.
M. J. G. Vilaça-Silva, *University of Minho, and M. Fernandes-Ferreira*

GENE TRANSFER TO PLANTS

P-2025 A GFP - BAR Construct for Switchgrass Transformation
J.K. McDaniel, *The University of Tennessee, H.A. Richards, H. Sun and B.V. Conger*

P-2026 Factors Affecting Increased Chromosomal Aberrations in Callus Cultures and Plants of Barley (*Hordeum vulgare* L.) during Transformation Process
H.W. Choi, *University of California, P.G. Lemaux and M.-J. Cho*

P-2027 Quantitation of Transgenes in Soybean (Glycine max L.) by Real Time PCR
M.A. Schmidt, *University of Georgia, and W.A. Parrott*

P-2028 Transgenic Tropical Maize with the cry1E Gene Presents Resistance to Fall Armyworm (*Spodoptera frugiperda* J. E. Smith)
N. Bohorova, *CIMMYT Int., M. Royer, R. Frutos, P. Estañol, M. Pacheco, Q. Rancón-Cruz, S. McLean, and D. Hoisington*

P-2029 Endosperm-specific GFP Expression Driven by Barley D-Hordein Promoter and Its Inheritance in Transgenic Barley and Wheat Plants
M.-J. Cho, *University of California, H.-K. Kim, H.W. Choi, B.B. Buchanan and P.G. Lemaux*

P-2030 GFP to Determine Transformation Patterns in Cotton
R.H. Smith, *Texas A&M Univeristy, S.H. Park, and M.G. Salas*

P-2031 Sonication-Assisted *Agrobacterium*-Mediated Transformation of Embryogenic Soybean Tissue and Subsequent Monitoring of Gene Expression with GFP
K.M Larkin, *The Ohio State University, and J.J. Finer*

P-2032 Hypocotyl-Based Generation of Transgenic Soybean and Progeny Analyses
L. Chen, *Mississippi State University, J. M. Tyler, and N. A. Reichert*

P-2033 Generation of Transgenic Creeping Bentgrass (*Agrostis palustris* Huds.) Plants from Mature Seed-derived Highly Regenerative Tissues
M.-J. Cho, *University of California, K.V. Le, D. Okamoto, and P.G. Lemaux*

P-2034 Transgenic Plants of Kentucky Bluegrass (*Poa pratensis* L.) Generated from Mature Seed-derived Highly Regenerative Tissues
M.-J. Cho, *University of California, C.D. Ha, and P.G. Lemaux*

P-2035 Identification of a Novel Constitutive Maize Promoter and Characterization of Its Expression in Transgenic Maize
S.M. Jayne, *Pioneer Hi-Bred International, Inc., D. Liu, K. Hagemann, M. Mitchell, and D. Rice*

P-2036 High Frequency Shoot Regeneration from Immature Embryo Culture in Sorghum
T. Hagio, *National Institute of Agrobiological Resources, and Y. Ohkawa*

P-2037 Transient Gene Expresson Studies in Juncus Accuminatus (Bull Rush)
S.D.Rogers, *Salem-Teikyo University, and K.S.Sarma*

P-2038 Production of Transgenic Wheat at CIMMYT: One Year of Data
A. Pellegrineschi, *CIMMYT, S. McLean, L. Velazquez, R. Hernandez, R.M. Brito, and D. Hoisington*

P-2039 Genetic Transformation of Elite Oat Cultivars
A.R. Carlson, *University of Wisconsin, and H.F. Kaeppler*

P-2040 Somatic Embryogenesis and Genetic Transformation of South African Sweetpotato Cultivars
C. L. Daniels, *Tuskegee University, M. Egnin and C.S. Prakash*

P-2041 Transformation of Synthetic Protein Gene into Vietnamese Sweetpotato Cultivars by *Agrobacterium tumefaciens*
D. T. Phong, *Institute of Biotechnology, P. B. Ngoc, M. Egnin, C.S. Prakash, and L.T. Binh*

PLANT TISSUE CULTURE

P-2043 Somatic Embryogenesis in *Limonium bellidifolium* (Gouan) Durmort. Plumbaginaceae
M.A.M.Aly, *University of Florida, and B. Rathinasabapathi*

P-2044 Development of Somatic Embryos of *Coffea arabic*: from One Cell to Cotyledonary Stage
V. M. Loyola-Vargas, *Centro de Investigación Científica de Yucatán, Quiroz-Figueroa F. R., and R. Rojas-Herrera*

P-2045 In Vitro Regeneration of Cowpea by Thidiazuron
L.E. Marsh, *Lincoln University*

P-2046 Patterns of Variation of *n*-alkanes During In Vitro Induction of Somatic Embryogenesis from Flax Hypocotyls (*Linum usitatissimum* L.)
A.C. Cunha, *University of Minho, and M. Fernandes-Ferreira*

P-2048 Regeneration of F1 Hybrids Derived From Crosses Between Cultivated Alfalfa and a Highly Regenerable Regen SY Line
J. Will, *University of Wisconsin Biotechnology Center, and S. Austin-Phillips*

P-2049 Shoot Organogenesis from Nodal Explants of Corn
M.M. Young, *Mississippi State University, and N.A. Reichert*

P-2051 Regeneration from Nine Maturity Groups of Soybean via Hypocotyl-Based Organogenic Regeneration
A.L. Woods, *Mississippi State University, L. Chen and N.A. Reichert*

P-2052 Effect of Different Auxin and Sugar Treatments on Callus Induction, Embryogenesis and Plantlet Regeneration from Mature Embryos of Wheat (*Triticum aestivum* L.)
M.G. Mendoza , *University of Wisconsin, and H.F.Kaeppler*

P-2053 Somatic Embryogenesis from Nucellar Tissue of Cashew (*Anacardium occidentale* L.)
V. Cardoza, *St Aloysius College, and L. D`Souza*

P-2054 Investigation of the Interspecific Hybrid Capsicum Baccatum Var. Pendulum (Wild.) Eshbaugh x C. Annuum L. Grown via Embryoculture Technique
R. Pandeva, *Bulgarian Academy of Sciences, V. Nikova, and R. Vladova*

P-2055 Cell Suspension Culture of *Persea pachypoda* and *P. cinerascens*
T. Witjaksono, *University of Florida, and R. E. Litz*

P-2056 Somatic Embryogenesis and Plant Regeneration from Cotton Anther Culture
B.H. Zhang, *Chinese Academy of Agricultural Sciences, R. Feng, F. Liu, and X.L. Li*

P-2058 Factors Affecting Induction of Somatic Embryogenesis in Velvetleaf (*Abutilon theophrasti*)
Y.L. Klein, *Andrews University, and D.A. Steen*

P-2059 Somatic Embryogenesis and Plant Regeneration in Typha Aungustifolia (Narrow Leaf Cattail)
S.D.Rogers, *Salem-Teikyo University, and K.S.Sarma*

P-2060 Analysis of Antibody-Binding Site of Rice Allergen RA17 with Human Monoclonal Antibodies
H. Shinmoto, *Tohoku National Agricultural Experiment Station, T. Kimura, K. Yamagishi and M. Suzuki*

MICROPROPAGATION

P-2061 In Vitro Propagation of Two Zizphus Species; Z. Spina christi and Z. Muratiana
S. Al-Mazrooei, *Kuwait University, and P. Ramos*

P-2062 Micropropagation of *Vaccinium cylindraceum* Smith (Ericaceae), an Azorean Endemic Species
M. J. Pereira, *University of Azores*

P-2063 Seed Germination and In Vitro Propagation of Sucupira Branca [*Pterodon pubescens (Benth.) Benth*], a Medicinal Plant
J.E.B.P.Pinto, *UFLA/DAG, M.C.F. Coelho, O.A. Lameira, E.J.A. Santiago and F.G. Silva*

P-2064 Multiplication Strategies for *Hypericum foliosum* Aiton, an Endemic Azorean Species
M. Moura, *Universidade dos Açores, and G. Belo*

P-2065 Chemically Induced Resistance of *Carica Papaya* and *Phytophthora Palmivora*
J.Y. Zhu, *Hawaii Agriculture Research Center, M. Fitch, S. Ferreira, and P. Moore*

P-2066 One-step, *In Vitro* Acclimatization of Carnation Using a Mist Reactor
M.J. Correll, *Worcester Polytechnic Institute, and P.J. Weathers*

P-2067 Development of Interspecific Hybrids in Oil Seed (*Brassicas (Cruciferae)*
V. Raja, *Indian Agricultural Research Institute, and I. Ahuja*

P-2068 Detection and Elimination of *Verticillium* Infections of Mint
N. Wang, *Oregon State University, and B. Reed*

P-2069 Optimization of an In Vitro Bioassay for Wheat Diseases
A. Pellegrineschi, *CIMMYT, M.M. Salgado, M. Mezzalama, S. McLean, and D. Hoisington*

P-2070 Cryopreservation of Temperate and Tropical Crops by Encapsulation-Vitrification Protocol
D. Hirai, *Hokkaido Plant Genetic Resources Center, and A. Sakai*

P-2071 Cryopreservation of Somatic Embryo of Papaya (*Carica papaya* L.) by Vitrification
H. Takagi, *Japanese International Research Center for Agricultural Sciences, and T.G.Lu*

SILENT ABSTRACTS

P-2072 In Vitro Propagation of Edible Ginger, *Zingiber officinalie* as Influenced by Bud Dormancy and Autotrophy
M.J. Tanabe, *University of Hawaii at Hilo, and K. Okuhara*

P-2073 Transgenic Kentucky Bluegrass (*Poa pratensis L.*) Plants Obtained by Microprojectile Bombardment of Embryogenic Callus
C. Gao, *DLF-Trifolium A/S, Research Division, and L. Hansen*

P-2074 Callus Induction and Organogenesis in Wheat Hybrids and Amphiploids
N. Tyankova, *Bulgarian Academy of Sciences, A. Dryanova, N. Zagorska, and D. Dimitrov*

P-2075 Plant Regeneration Through Somatic Embryogenesis from Leaf Tissue of *Odina wodier* Roxb., a Tropical Tree
S. Pandey, *Banaras Hindu University, and V.S. Jaiswal*

P-2076 In Vitro Assay of 2,4-D Resistance Transgenic Cotton
B. H. Zhang, *Chinese Academy of Agricultural Sciences, Hong-Mei Wang, Fang Liu, Yun-Hai Li and Zheng-De Liu*

P-2077 Role of Suspension Culture in Scale-up of Somatic Embryogenesis in Mango (*Magifera indica* L.) cv. Amrapali
H. Ara, *Banaras Hindu University, U. Jaiswal, and V.S. Jaiswal*

P-2078 Plant Regeneration Through Somatic Embryogenesis in Root Derieved Callus of Elephant Tusk Cactus (Coryphantha elephantidens (Lem.) Lem.)
B. S. Bhau, *University of Delhi, and A. K. Wakhlu*

Vertebrate/Toxicology Posters

SUNDAY, JUNE 11	MONDAY, JUNE 12	TUESDAY, JUNE 13
10:00-21:00	10:00-21:00	10:00-13:00

Posters mounted Saturday, June 10, 15:00 – 17:00 hrs.
Posters must be removed from Exhibit Hall by 14:00, June 13.
Authors will be present at their posters the following days and times:

SATURDAY, JUNE 10	SUNDAY, JUNE 11	MONDAY, JUNE 12	TUESDAY, JUNE 13
All Authors Present	Even Authors Present	Odd Authors Present	All Authors Present
19:30 – 20:30	13:00-13:30	13:00-13:30	12:00-12:30

BIOTECHNOLOGY AND CANCER BIOLOGY

VT-2000 Assay Validation for Mycoplasma Screening Using Gen-Probe MTC-NI Detection System
S. Nadolski, *Genetics Institute, J. Demers, M. Griffin, and P. Ozker*

VT-2001 Hydrostatic Pressure Induced Interleukins and Cytokines Production by Normal Human Dermal Fibroblasts
S. Koyama, *Japan Marine Science and Technology Center, S. Fujii, and M. Aizawa*

VT-2002 Selective Induction of G-CSF in Macrophages by a Stimulatory Monoclonal Antibody: A New Signal Gateway Potentially Useful in Cytokine Therapy
Y. Aoki, *Japan Tobacco Inc., S. Sha, H. Mukai and Y. Nishi*

VT-2003 Caco-2 High Throughput Screening Technologies: Pre-Grown Caco-2 24 Well Plate Product and the Use of a HTS Assay as a Rapid *In Vitro* Screen for Predicting Intestinal Drug Absorption
Brenda F. Kahl, *Trega Biosciences / Navicyte Inc., K. Click, Y. H. Lee, and S. R. Slivka*

VT-2004 Selective Expansion and Non-radioisotopic Assay of Human Natural Killer Cells
S. Watanabe, *RIKEN (Inst. Physical. & Chem. Res.) Cell Bank, H. Harada, K. Saijo, and T. Ohno*

VT-2006 Two-dimensional Cell Blot Method
T. Terasaki, *National Cancer Center Research Institute, Z. Yamaizumi, and K. Tanaka*

VT-2047 Effective Cell-Free Translation System Based on 5'NCR of L-A Virus mRNA
T. Ebihara, *Tokyo Institute of Technology, E. Kobatake, and M.Aizawa*

Vertebrate/Toxicology Posters

EXTRACELLULAR MATRIX

VT-2007 Role of Matrix Metalloproteinases i9n Alpha-1-Proteinase Inhibitor Degradation by Neutrophil-Derived Proteases
W.J. Bellucci, *State University of New York at Stony Brook, E.J. Roemer, C.L. Ren, and S.R. Simon*

VT-2008 Evaluation of the ComPosition of the Extracellular Matrix Synthesized by Human Prostate Stromal Cells in Culture
E. Scotto-Lavino, *State University of New York at Stony Brook, H.A. Sawka, S.R. Simon and E.J. Roemer*

VT-2009 Type I Collagen Gene Expression in Human Uterine Leiomyoma Cell Lines
T. Wallace, *Spelman College, S. McClure, E. Mack, J. Barlow, and J. Crowe*

VT-2010 Enhanced Matrix Metalloproteinase (MMP) -2 Activity in Gingival Fibroblasts from Down Syndrome Patients
T. Komatsu, *Kanagawa Dental College, M. Furue, T. Kubota, A. Miyagi and E. Kubota*

GROWTH, SENESCENCE, APOPTOSIS, AND DIFFERENTIATED FUNCTION

VT-2011 Protein Kinase C Functions in TGF-beta-Induced Cellular Senescence Programs
Y. Katakura, *Kyushu University, Y. Tabira, T. Miura, N. Uehara and S. Shirahata*

VT-2012 Hyperoxia Induces the Neuronal Differentiated Phenotype of PC12 Cells via a Sustained Activity of Mitogen-Activated Protein Kinase Induced by Bcl-2
S. Katoh, *University of Tokyo Hospital, Y. Mitsui, K. Kitani and T. Suzuki*

VT-2013 Participation of Bcl-2 in Cellular Life Span
M. Sasaki, *Hiroshima University T. Kumazaki, M. Nishiyama, H .Takano, and Y. Mitsui*

VT-2014 Subtractive Screening of Genes Involved in Cellular Senescence
N. Uehara, *Kyushu University, Y. Katakura, T. Miura, and S. Shirahata*

VT-2015 Characterization of the 5'-Flanking Region of Human Telomerase Reverse Transcriptase Gene
T. Miura, *Kyushu University, Y. Katakura, S. Nishimura, Y. Tabira, Y. Kotake, N. Uehara, K. Tashiro and S. Shirahata*

VT-2016 Expression of Urokinase-type Plasminogen Activator mRNA by IL-1beta and TNF-alpha in Human Lung Microvascular Endothelial Cells
K. Kobayashi, *Tokyo Medical University , K. Takahashi, H. Yagyu, T. Morisako, K. Kishi, R. Mikami, O. Watanabe, S. Oh-ishi, H. Nakamura, T. Hashimoto, and T. Matsuoka*

VT-2017 Expression of MHC Antigens and Adhesion/Costimulation Molecules of Dendritic Cells from Human Blood During their Differentiation *In Vitro*
M. Chiriva-Internati, *Arkansas Cancer Research Center, UAMS, F. Grizzi, G. Ceva-Grimaldi, and N. Dioguardi*

VT-2048 Developmental Changes in Expression of Beta-Adrenergic Receptors in Cultures of 2C12 Skeletal Muscle Cells
Ronald B. Young, *Marshall Space Flight Center, K.Y. Bridge, and J.R. Vaughn*

Vertebrate/Toxicology Posters

IN VITRO TOOLS AND TECHNIQUES

VT-2018 Cytotoxic Effects of Chemically Modified Tetracyclines on R22 Cells
K. Guilfoy, *State University of New York at Stony Brook, S. R. Simon and E. J. Roemer*

VT-2019 Biochemical Changes Induced in Human Cells by the Vesicating Agent Sulfur Mustard
W.J. Smith, *US Army Medical Research Institute of Chemical Defense, O.E. Clark, F.M. Cowan, M.E. DeJoseph, and C.L. Gross*

VT-2020 Phototoxicity Testing Using 3-D Reconstructed Human Skin Models
P.A. Jones, *Unilever Research Colworth, A.V. King, and L.K. Earl*

VT-2021 Recuperative Effect of Fermented Milk, Kefir to UV-Damaged Cultured Cells
K. Teruya, *Kyushu University, T. Nagira, J. Narisawa, K. Kusumoto, Y. Katakura, D. W. Barnes, S. Tokumaru, and S. Shirahata*

VT-2022 Effects of Chemical Inhibitors on Protein Tyrosine Phosphorylation and Cytotoxicity
Wendy Scholz, *Nalge Nunc International Corporation, and S. Moy*

VT-2023 The Spontaneously Contracting Primary Cultures of Neonatal Rat Cardiomyocytes as an In Vitro Toxicity Screening System
U. Schramm, *Novartis Pharma AG, M.D. Estevez, B. Greiner, W. Frieauff, and A. Wolf*

VT-2024 Predictive Value of In Vitro Chemosensitivity Test Using the 3-Dimentional Collagen Gel Droplet Culture Method in Recurrent Non Small Lung Cancer
H. Kobayashi, *Research & Development Nitta Gelatin Inc.*

VT-2025 In Vitro Cytotoxicity of S-Nicotine and S-Cotinine as Determined by the FRAME's Neutral Red Uptake (NR) and Kenacid Blue (KB) Assays
J. Oey, *Institut fuer biologische Forschung, and E. Roemer*

VT-2026 Induction of Secondary Light Chain Gene Recombination in Human Plasma Cells by Caffeine is Independent from Both the Upregulation of RAG Proteins Expression and Germ-Line Transcription
H. Tachibana, *Kyushu University, T. Chiwata, Y. Nagahiro, H. Haruta, and K. Yamada*

VT-2027 Acute and Chronic Levels of Cytotoxicity and Immuno-stimulation for 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), a Polymer Catalyst, Using In Vitro Modeled Assays
A.M. Wright, *Mercer University, J.G. Strom, and M.E. Rothenberg*

VT-2028 Inhibitory Effect of Sialyloligosaccharide and Their Derivatives on Adhesion of Entero-Pathogenic Bacteria to Human Intestinal Epithelial Cells
Y. Sugita-Konishi, *Taiyo Kagaku Company, Ltd., S. Sakanaka, K. Sasaki, and F. Amano*

VT-2029 Age and Ocular Irritancy as Measured In Vitro
J.G. Sivak, *University of Waterloo, K.L. Moran, and D.G. Dixon*

VT-2046 Measurement of Immune Response In Mice Coinfected With Babesia Microti and Borrelia Burgdorferi Using Enzyme Linked Immunoflourescence Assay and Immunoflourescence Assay
C. D. Thill, *State University of New York at Stony Brook, D. LiVine, and J. L. Benach*

Vertebrate/Toxicology Posters

NEW TECHNOLOGIES

VT-2030 New Colorimetric Method for *In Vitro* Cell Number Estimation Using Carmine as a Specific Chromosome Dye
T. Garcia-Gasca, *Universidad Autónoma de Querétaro, V. Paz-González, M.C. Moncada-Alvarez, and L.A. Salazar-Olivo*

VT-2031 Authentication, Amelogenin, and Sex in Human Cell Lines
Y. A. Reid, *American Type Culture Collection, S. Durkin, and D. Boles*

VT-2032 The Choice of Vehicle Solution for Cryoprotectants Impacts Cell Viability After Exposure at Low Temperatures
L.H. Campbell, *Organ Recovery Systems, Inc., R.N. Rutledge, M.J. Taylor, and K.G.M Brockbank*

VT-2033 Development of Protein-Free Media for High Density Suspension Culture of HEK 293 Cells and Recombinant Adenovirus
C.J. Card, *HyClone Labs, Inc., and B.B. Barnett*

VT-2034 The Development of Serum Free Media for Vaccine Production in Vero Cells
G. W. Reese, *HyClone Laboratories, Inc., B. B. Barnett, and C. J. Card*

VT-2035 Cell Growth and Differentiation on Chemically Modified Surfaces
W.K.Scholz, *Nalge Nunc International, and S.K.W. Nanda*

VT-2036 Successful Transformation of Cryopreserved Lymphocytes: A Resource for Epidemiological Studies
J.C. Beck, *Coriell Institute for Medical Research, C.M. Beiswanger, E. John, E. Satariano, and D. West*

VT-2037 Development of Cell Culture System from Liver, Kidney and Spleen of African Catfish *Clarias gariepinus*
G. S. Kumar, School of Environmental Studies, I.S.B. Singh, and R. Phillip

VT-2038 Efficient Isolation and Long-Term Viability of Bovine Small Preantral Follicles In Vitro
T. Itoh, *Research Institute for the Functional Peptides, H. Abe, M. Kacchi, and H. Hoshi*

VT-2039 The Culture of Rat vibrissa Cells Using Low-serum Medium
H. Takahashi, *Toyobo Co., Ltd., and T. Ishibashi*

VT-2040 The Establishment of Human T-T Hybridomas Producing IL-4 and IFN-Gamma
M. Maeda-Yamamoto, *The National Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries, K. Kawamoto, M. Nagase, H. Kawahara, and K. Hakamata*

SILENT ABSTRACTS

VT-2041 Inhibition of Calpain Increases Cell Migration and Wound Healing via a Protein Kinase C Dependent Pathway in Human Dermal Microvascular Endothelial Cells
L.L. Chiu, *Stanford Medical School, and M.A. Karasek*

Vertebrate/Toxicology Posters

VT-2042 Morphological Survival and In Vitro Maturation of Immature Bovine Oocytes Exposed to EGTA With or Without Cryopreservation
M.R. Blanco, *Universidad Nacional de Lomas de Zamora, L. Simonetti, and P. Palermo*

VT-2043 The relationships Between Thrombin-Induced Nitric Oxide Production and Intracellular Calcium Concentrations in Bovine Endothelial Cells
Y. Minai, *Tamagawa University, N. Takadera, M. Furusho, A. Higa, T. Hidaka, Y.* Matsuoka, and M. Haga

VT-2044 The Effects of Multiple Forms of Vanadate on Sugar Transport in Human Fibroblasts
R. J. Germinario, *SMBD-Jewish General Hospital , McGill University, S.P. Colby-Germinario, K. Nahm, and B. Posner*

VT-2045 Demonstration of Membrane-Bound Carbonic Anhydrase II in Human Cancerous Pancreatic Duct Cells (CAPAN-1 line)
L. Alvarez, *Université Paul Sabatier, M. Fanjul, and E. Hollande*

PS-1

The Vanishing Life of Earth: Do We Really Care? Peter Raven. Missouri Botanical Gardens, Engleman Professor of Botany, PO Box 299, Saint Louis, MO 63166-0299

An estimated 5–7 million species of plants, animals, and microorganisms, only a quarter of them known to science, are disappearing so rapidly that it appears we are entering the sixth great extinction event in the history of life on Earth. We base our civilization these organisms, which alone have the ability to capture a small fraction of the Sun's energy and transform it into chemical bonds—a strategy allowing the gradual expenditure of the energy and the maintenance of all life on our planet, including our own. Since we depend on these organisms individually for the sustainable production of our food, most of our medicines, and a great deal of our shelter and clothing; collectively for the ecosystem services that sustain our habitats; and for moral and spiritual reasons as well, we must regret losing so many of them before we even recognize that they are here. Our runaway population growth, high levels of consumption (in certain regions), and use of inappropriate technology are the forces driving the loss of these species and at the same time making global sustainability so difficult to achieve. Strenuous efforts will be needed to preserve the most complete possible set or organisms for the future, thus observing Aldo Leopold's famous dictum, The first rule of intelligent tinkering is to save all the cogs and wheels.

PS-2

Advances in Understanding the Molecular, Genetic and Protein Structure of Neurodegenerative Disease. Stanley B. Prusiner. University of California, San Francisco, CA 94143-0518.

Prions are infectious proteins causing neurodegenerative diseases including Creutzfeldt-Jakob disease (CJD) of humans as well as bovine spongiform encephalopathy and scrapie of animals. The human prion diseases may be manifest as sporadic, genetic, or infectious disorders. An abnormal isoform of the prion protein (PrP), which is encoded by a chromosomal gene, is the major, and most probably the only, component of the prion. The cellular isoform PrPC has a high? -helical content, whereas the pathogenic isoform PrP^{Sc} acquires a substantial? -sheet content posttranslationally. Conversion of PrP^{C} into PrP^{Sc} occurs in rafts or caveolae-like domains (CLDs) near the cell surface and seems to involve an auxiliary factor designated protein X. During prion propagation, protein X appears to bind to the C-terminus of PrP^{C} but not to the product of the conversion process, PrP^{Sc}. Diversity of prions as manifest by differences in neuropathology and incubation times seems to be enciphered within the conformation of PrP^{Sc}. Besides pathologic prions, which are found in mammals, non-pathologic prions have been identified in fungi; it is likely that many other prions will be found as the search intensifies.

JS-1

Functional Food for Prevention of Allergy. S. KAMINOGAWA. Department of Applied Biological Chemistry, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657 JAPAN. Email: akamino@mail.ecc.u-tokyo.ac.jp

In Japan, allergic disease affects 30% of the population. Therefore, treatment of allergic disease is required. We have attempted to establish a good model of allergy and to survey the types of food materials useful as functional foods. A transgenic mouse that we developed, which has only one kind of T cell antigen receptor, shows a high level of IgE in serum following oral administration of an allergen. Furthermore, we could find signs of the destruction of intestinal villi and were able to confirm the release of histamine in this model. These results show that this transgenic mouse is possibly a good model of allergy. In this transgenic mouse, we investigated the effect of administration of several types of materials, such as probiotics, nucleotides, and peptides derived from allergens, on the production of IgE. We were able to demonstrate that a non-pathogenic microorganism, *L. casei*, could inhibit antigen-induced IgE production through induction of IL-12 secretion. We found that supplementation of the diet with nucleotides down-regulated the serum IgE. Furthermore, we succeeded in suppressing the allergic reaction by means of amino-acid substituted analogs of allergen-derived peptides.

JS-2

Regulation of Permeability in Intestinal Epithelium by Food Components. M. Shimizu. Department of Applied Biological Chemistry, The University of Tokyo, Tokyo 113-8657, Japan. E-mail: ams316@mail.ecc.u-tokyo.ac.jp

Water-soluble low-molecular weight substances which do not have specific transporters are thought to be absorbed mainly by passive diffusion between the cells in the intestinal epithelium. Recent studies have demonstrated that this pathway (paracellular pathway) is more important than had been previously believed, because many bioactive hydrophilic substances in food, such as oligopeptides and phytochemicals, are probably absorbed via this pathway. The paracellular permeability is regulated by tight junctions (TJ). If the paracellular pathway is increased by modulating the TJ structure, the efficiency of absorption of water-soluble functional substances would be increased. We have searched for TJ-modulatory substances in food materials by using a human intestinal Caco-2 cell monolayer system. Among more than 100 samples tested, extracts of sweet pepper, horse radish and some mushrooms were observed to increase the TJ-permeability. The active substance in sweet pepper was identified to be capsianoside, a diterpene glycoside. This substance is likely to be incorporated into the cell membrane and associated with intracellular actin filaments, thereby altering TJ structure. Another active component isolated from the mushroom, *Flammulina velutipes*, was identified to be a protein of 31 KD. The formation of a small pore in the cell membrane by this protein is probably the cause of the TJ opening. Recently we have observed that a fraction prepared from milk protein hydrolyzates had TJ-modulatory activity. Thus the intestinal absorption via the paracellular route could be useful for the development of a new functional food, in which the increased intestinal absorption of bioactive substances is intentionally designed.

JS-3

Functional Peptides Derived from Proteins in Foods. M. YOSHIKAWA. Research Institute for Food Science, Kyoto University, Uji, Kyoto 611-0011, JAPAN. Email: yosikawa@food2.food.kyoto-u.ac.jp

Many kinds of bioactive peptides are released from proteins which have not been regarded as precursors for them. These are classified as follows: 1) ligands for receptors, 2) enzyme inhibitors, 3) regulators of intestinal absorption, 4) antimicrobial peptides, 5) antioxidative peptides, 6) others. Usually specific activities of such peptides are smaller than those of endogenous bioactive peptides. However, some of them show physiological effects after the oral administration while endogenous peptides are almost inactive by oral route. We isolated two types of antihypertensive peptides from enzymatic digests of animal proteins. Inhibitors for angiotensin-converting enzyme (ACE) derived from fish protein was activated by ACE itself and exhibited long-lasting effect. We named it prodrug type ACE inhibitor. We isolated two endothelium-dependent vasorelaxing peptides from ovalbumin digest, both of which exhibited antihypertensive activities at 10mg/kg po. We also obtained low molecular weight peptides showing hypo-cholesterolemic activity after the oral administration in mice without increasing fecal excretion of cholesterol and bile acids. We isolated a peptide stimulating phagocytosis by human polymorphonuclear leukocytes from soybean protein digest. This peptide binds to fMLP receptor though itÕs not formylated. Interestingly, this peptide prevented alopecia induced by cancer chemotherapy agent etoposide. IL-1 seems to be involved in the mechanism for anti-alopecia because it was blocked by an IL-1 blocker.

JS-4

Antioxidative Plant Foods for Disease Prevention. TOSHIHIKO OSAWA, Nagoya University Graduate School of Bioagricultural Sciences, Chikusa, Nagoya 464-8601, Japan. Email: osawat@agr.nagoya-u.ac.jp

Oxidative stress may cause free radical reactions to produce deleterious modifications in membranes, proteins, enzymes and DNA. Age-related diseases such as cancer, atherosclerosis and diabetes are supposed to be correlated with oxidative stress although the dtailed mechanisms are still unclear. Our research group has been involved in developing novel ELISA methods by application of immnochemistry. We have developed many types of monoclonal and polyclonal antibodies which are specific to oxidatively damaged DNA base such as 8-OH-deoxyguanosine and lipid peroxidation products including lipidhydroperoxides, malondialdehyde, 4-hydroxynonenal and aclorein etc. By monitoring these oxidatively damaged products as biomarkers, we have been screening many different types of dietary antioxidants. From our hypothesis that endogenous antioxidants in plants must play an important role for antioxidative defense systems from oxidative stress, an intensive search for novel type of natural antioxidants has been carried out from numerous plant foods, and we have isolated and identified a number of lipid-soluble and water-soluble dietary antioxidants. Recently, we have isolated lipid-soluble antioxidative lignans, such as sesaminol, sesamolinol, pinoresinol and p1 from sesame seeds. We also found that sesaminol can be formed from sesamolin during the refining process of sesame oil and also from sesaminol glucosides (SG), present in the defatted sesame flour after (DSF) stripping off oils from sesame seeds. Recently, we found that sesaminol inhibited strongly oxidative modification of apoB protein of low-density lipoprotein induced by 2,2'-azobis (2,4-dimethylvalero-nitrile) and antioxidative mechanism has been examined in details. These background prompted us to evaluate antiatherogenic activity of sesaminol and its precursors (SG) by *in vivo* system using high colesterol fed rabbits by feeding DSF which contains about 1% SG. From this result, we have found that SG inhibits atherosclerotic vascular disease in cholesterol-fed rabbits and suggested that sesaminol directly protects LDL from oxidation by acting as a lipid soluble antioxidant. Recently, we have also examined antioxidative effect of SG in Watanabe Heritable Hyperlipidemia (WHHL) rabbits. The percentage area of aorta covered with plaque in the SG-treated rabbits was reduced compared to the control, and it was showed that lipid peroxide was decreased significantly and also significant increase in the activity of glutathione peroxidase and glutathione S-transferase in tissues including liver and aorta. This study indicates that the reduction of atherosclerosis by SG-treatment relies not on its cholesterol lowering effect but more heavily on its antioxidant potential to inhibit LDL oxidative modification in WHHL rabbits. In the course of our investigation to find novel type of antioxidative substances in the plant materials, most natural lipid-soluble antioxidants can be classified to two different types: phenolic and&#61472Ǝta-diketone type antioxidants. However, there have not been found the antioxidative substances which has both functional groups; phenolic and beta-diketone groups in the same molecule. In order to a new type of antioxidative compounds which has both phenolic and beta-diketone moiety in the same molecule, the authors forcused on curcumin, main yellow pigments of *Curcuma longa* (turmeric). Curcumin has been used widely and for a long time in the treatment of sprain and inflammation in indigenous medicine, however, there is some limitation to utilize curcumin for food and medicinal purposes because of its yellow color. Recently, we succeeded in obtaining a strong lipid-soluble antioxidant, tetrahydrocurcumin(THC), by hydrogenation using Pd-C (or Raney-nickel) as the catalyst. Recently, we tried many biological activities of THC using a wide variety of evaluation systems. THC was found to be produced from curcumin during absorption from the intestines, and more potent antioxidant than curcumin. Recently, we found that THC is a promising chemopreventive agent than curcumin in the 1,2-dimethylhydrazine (DMH) induced mouse colon carcinogenesis model. Detailed antioxidative mechanism of THC will be discussed.

JS-5

Prospects on Development of Physiological Functional Foods Kazuki Shinohara. National Food Research Institute. MAFF, Tsukuba, Ibaraki, 305-8642 Japan E-mail: kazuki@nfri.affrc.go.jp

At present, our daily diet is becoming satisfactory in term of quantity. However, with change of food habits, food-related diseases such as cancer, allergy, obesity and geriatric diseases are increasing in Japan. Furthermore, the aged population is expected to reach a maximum in the next century. From these points of view, the demands of consumer are rapidly rising toward the high quality of foods providing well-balanced nutrients, rich tastes and health promotion. In order to response to the needs for improved foods, it is important to elucidate the physiological functions of food components. National projects carried out by university groups and the Ministry of Agriculture, Forestry and Fisheries on the physiological functions of foods revealed that foods have functions controlling homeostasis in our body, as well as nutritional and sensory functions. From the projects above, various physiological functions of components of vegetables, soybean, milk and other foods or their metabolites have been discovered, such as anticarcinogenicity, antioxidativity, immunoactivity, endocrinomodulation, hypotensive effect, cholesterol control, intestinal control, and etc. Among the food components, the physiological functions of 1) dietary fibers, 2) oligosaccharides, 3) sugar-alcohols, 4) polyunsaturated fatty acids, 5) peptides and proteins, 6) glycosides, isoprenoids and vitamins, 7) alcohols and phenol, 8) choline, 9) lactobacteria, 10) minerals and 11) others (fermented vinegar, prebiotics) are paid attention. The projects above exerted a great influence on the food industries and the Ministry of Welfare and Health (MWH) leading to the introduction of the terminology of Functional Foods. Functional foods were defined as a food including the compounds with following criteria ; 1) clear effectiveness for specified health, 2) chemical structure, 3) clear mechanisms at cellular level, 4) evident effectiveness by oral administration, 5) safety, 6) stability in foods, 7) acceptability as food, and 8) variety of food styles. The member companies of the Japan Healthy and Nutritional Food Association collaborate in the working groups which are responsible for collecting scientific evidence, considering 11 identified categories of functional components as stated above. In 1991, a system for licensing the Foods for Specified Health Use(FSHU) was established by MWH. A FSHU is a food which is, based on the knowledge concerning the relationship between foods or food components and health, expected to have certain health benefit, and has been licensed to bear a label claiming that a person who uses it for specified health use may expect to obtain the health use through the consumption thereof. To obtain a license for a FSHU, the following requirements should be necessary; 1) Contribution to the improvement of dietary habits and the maintenance and enhancement of health should be expected. 2) A medical and nutritional data on specific health aspects. 3) Appropriate amount of consumption of the food or relevant compound based on medical and nutritional basis. 4) Safety data of the food or relevant compound based on experience, 5) Physicochemical properties, test methods and methods of qualitative and quantitative determination, 6) Data on nutritional composition. The composition of the product should not be defective in comparison with the composition of nutritive components which are normally contained in similar types of foods. In addition to these requirements, the product should be of a kind of foods that are consumed in ordinary dietary patterns, rather than those consumed only occasionally. The product should be in a form of ordinary foods, other than pills, capsules, etc. The official permission for licensing FSHU is under control of the MHW. In the system for licensing, overseas applicants need to apply directly to the MHW (office of Health Policy on Newly Developed Foods, Environmental Health Bureau). Allergen-free rice and low phosphate content of milk L.P.K. were approved by MWH as the first FSHU. About 167 kinds of FSHU containing dietary fibers, oligosaccharides, lactobacteria and others is now licensed.

JS-6

Principles learned from how marine organisms osmoregulate: Applications to biomedical research. J.D. FERRARIS Laboratory of Kidney and Electrolyte Metabolism, National Heart Lung Blood Institute, National Institutes of Health, Bethesda, MD 20892. E-mail: jdf@helix.nih.gov

The principles learned from studying osmoregulation in marine animals have led us to an understanding of how human kidney cells tolerate the osmotic stress to which they are constantly exposed. Aquatic organisms live in salt-variable habitats and have permeable tissues that are in direct contact with their environment. This makes osmoregulation necessary and also makes marine organisms valuable models in biomedical research. Renal medullary cells, among mammalian cells, are uniquely exposed to hyperosmotic stress; the study of how these cells osmoregulate, in particular, has benefitted from the principles learned from marine organisms. With the exception of the halobacteria, the cells of all organisms have the same adaptive response to a high salt environment, *i.e.*, the accumulation of intracellular organic osmolytes which acts to conserve macromolecular function. This principle evolved from the 1952 discovery by Florkin of intracellular accumulation of amino acids with increased salinity in the Chinese mitten crab, *Eriocheir sinensis*. Bacteria, yeast, plants and animals accumulate organic osmolytes which group into only a few chemical categories: polyols, methylamines, and amino acids. Additionally, hyperosmotic accumulation of organic osmolytes is transcriptionally regulated in bacteria, yeast, plants and mammals. These facts bring up the obvious question of whether there is some highly conserved mechanism that all of these organisms have in common. Alternatively, the similarity in adaptive response could be a matter of convergence. The hypothesis that there is a conserved mechanism led to the discovery of an osmotic response element (ORE) in the flanking region of the aldose reductase gene; the gene responsible for the adaptive accumulation of sorbitol during high salt stress in renal cells. Since then, several mammalian OREs have been discovered. Sequence containing an ORE was identified for the canine Na- and Cl-coupled betaine transporter gene as well as the Na/myo-inositol cotransporter gene. Because it is possible to find homology between the OREs of the AR genes and those of the betaine and inositol genes, a consensus for the mammalian ORE was derived by functional assessment. Most recent studies have yielded discovery of other *cis*-elements that potentiate the ORE response and a *trans*-activating factor that binds to the ORE.

JS-7

The Egg and I: Unique Insights from Studies on Embryos of Marine Organisms. DAVID EPEL. Hopkins Marine Station of Stanford University, Pacific Grove CA 93950. Email: depel@stanford.edu

Marine organisms provide easily studied models of cell processes that provide insights relevant to all organisms, from jellyfish to man. One of the best studied models are gametes of sea urchins, worms clams and squid. Studies on the fertilization process in sea urchins were the first to unravel how the sperm activates the egg to begin development; the stimulus is a transient rise in calcium ion. Similar mechanisms have since been found to occur in the more difficult to study mammalian egg. Studies on sea urchins later revealed how this calcium increase occurs, and led to the discovery of an unexpected mode of calcium release which has since been found to operate in many processes in higher organisms, such as regulation of heart rate and sugar levels in blood (such knowledge of course relevant to understanding heart disease and diabetes). Recent work details the nature of sperm factors which activate the egg and are the basis for assisted reproduction technologies relevant to in vitro fertilization and even embryo cloning. Other studies on how embryos handle stress in the aquatic environment illuminate novel mechanisms for avoiding DNA damage, effects of toxins and even new modes of pathogen defenses.

JS-8

Dynamic Modulation of Neurons and Networks: Lessons from Lobsters and Crabs. E. MARDER. Volen Center and Biology Dept., Brandeis University, Waltham, MA 02454. Email: Marder@brandeis.edu

The stomatogastric ganglion of lobsters and crabs contains thirty neurons and generates two rhythmic motor patterns that control movements of the foregut. Modulatory neurons bring a large number of different amines and neuropeptides into the stomatogastric ganglion where they alter the intrinsic properties of neurons and the strengths of the synaptic connections among them. In so doing, defined modulatory inputs reconfigure the networks of the STG to produce a variety of different, behaviorally relevant outputs. Adult neurons and networks must maintain stable outputs despite continual turnover of their ion channel proteins. We use computational models and experimental work in adult and developing animals to argue that activity allows neurons to constantly regulate the density of their ion channels to maintain constant physiological properties. Research supported by NS17813 and MH46742.

JS-9

Biosilicification in Sponges and Its Application for *In Vitro* Synthesis of Polysiloxanes. K. SHIMIZU and D. E. Morse. Department of Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, CA 93106. Email: shimizu@lifesci.ucsb.edu

Silicon dioxide is the most abundant constituent of the Earth's crust, and is contained in wide range of inorganic materials. Organisms including diatoms, higher plants, sponges, produce silicon dioxide in the form of silica. The unique characteristics of biological silicas include their striking range of exquisite architectures, with precise genetic control from nanoscopic to macroscopic scales, and their synthesis under mild physiological conditions that contrast dramatically with the conditions required for the geological and industrial synthesis of silica. Elucidation of the mechanisms of biosilicification thus may lead to the development of new routes to the synthesis of silica and silicon containing materials under environmentally benign conditions. Sponges produce hydrated amorphous silica in the forms ranging from needle or fiber-like spicules. The silica spicules each contain an axial filament of protein. We discovered that the protein filaments from the spicules of the marine sponge *Tethya aurantia* can be dissociated into three isomeric proteins named silicatein alpha, beta and gamma. Analysis of the cDNAs encoding silicatein alpha and beta revealed that these proteins are members of the papain-like cysteine protease superfamily. Recombinant silicatein alpha exhibits Michaelis-Menten activity with tetraethoxysilane as substrate, catalyzing hydrolysis and subsequent condensation to form silica. Studies of homologs produced by site-directed mutagenesis confirmed identification of the active-site residues, permitting the design and synthesis of biomimetic structure-directing catalysts. Silicateins also promote synthesis of polysiloxanes containing silicon-linked methyl or phenyl groups, indicating a new catalyst for synthesis of silica and related compounds.

JS-11

On Earth and In Space: Insect Cell Glycosylation Pathway. H.A. WOOD*, M.L. Shuler and L. Joshi*, *Boyce Thompson Institute and Dept. of Chemical Engineering, Cornell Unversity, Ithaca, NY 14853. Email: HAW5@CORNELL.EDU

Investigations were conducted to evaluate the potential for insect cells to produce mammalian-type complex N-linked glycosylation of human proteins with the baculovirus expression vector system. Fidelity in glycosylation is important because glycosylation can control specific activity, solubility, thermal stability, antigenicity, resistance to protease digestion, etc. In the absence of terminal sialic acid residues on the oligosaccharides, the circulatory half-life of glycoproteins can be very short due to binding to asialoglycoprotein receptors. The current paradigm is that insect cells cannot perform sialylation. Previous investigations of N-linked glycosylation of secreted human-placental, alkaline phosphatase (SEAP) glycans produced in a variety of insect larvae and cell cultures indicated that 100% of the glycans had only terminal mannose residues, representing limited glycan processing. However, marked alterations in the structures and relative concentrations of N-linked SEAP glycans were observed when different baculovirus species, insect cell lines, cell culture media and culture conditions were employed. These changes resulted in a shift from 0% hybrid/complex glycans to 63% hybrid/complex glycans. More importantly, when cells were culture under condition of simulated microgravity, 20% of the SEAP glycans had terminal sialic acid residues. Accordingly, insect cells do have the entire metabolic pathway to produce pharmaceuticals that require human-type complex N linked glycosylation.

JS-12

In Vitro Production of Baculoviruses for Pest Control: from Insect to Formulated Bioinsecticide. R.C.L. MARTEIJN, C.D. de Gooijer, D.E. Martens and J. Tramper. Food and Bioprocess Engineering group, Wageningen University, PoBox 8129, Wageningen, The Netherlands. Email: rolf.marteijn@algemeen.pk.wag-ur.nl

The baculovirus is a very potent agent for pest control. To develop a bioinsecticide based on in-vitro produced baculovirus several hurdles have to be taken. The BacSAFT project, an EU funded project with several partners all over Europ, aims at developing an in-vitro baculovirus-based formulated bioinsecticide. Insects and viruses are isolated in Spain, cell lines are developed in Germany, virus is selected in The Netherlands and Germany, baculovirus is produced in-vitro in The Netherlands, safety-tested in Germany, formulated in the United Kingdom and finally evaluated in field trials at several european locations. An overview of this project will be given. The whole of the process will be taken into account with emphasis on the engineering features. Subjects discussed range from cell- line stability and virus choice, via the scale-up trajectory and the bioreactor type, to the viral passage effect, down stream processing and formulation. Results from baculovirus production at pilot scale (70 liters) will be shown. The engineering hurdles that need to be tackled will be pointed out as well as methods to optimise these parts of the process, including modelling and bioprocess designing.

JS-15

Acquisition of Host Proteases by Borrelia. JORGE L.BENACH. Department of Pathology, State University of New York at Stony Brook, Stony Brook, NY, USA, 11794-8691. E-mail: jbenach@path.som.sunysb.edu

Bacterial proteases have been associated with invasiveness and virulence. Some bacteria produce potent proteases that can degrade extracellular matrix macromolecules, and most bacteria can borrow proteolytic activity from their hosts. An increasing number of pathogenic bacteria can bind host-derived plasminogen which can be activated to plasmin on the bacterial surfaces by either endogenous plasminogen activators, (as in the case of gram positive cocci, and Yersinia), or by host-derived plasminogen activators. Although the genome of Borrelia burgdorferi has shown the presence of genes encoding for protease homologs, their numbers are low compared to other bacteria. For this reason, Borrelia may be more dependent on borrowed proteolytic activity than other bacteria that produce a larger protease repertoire. In vitro studies have shown that Borrelia can bind plasminogen via its lysine binding sites. This binding is of high affinity as may be expected from an organism whose median isoelectric point for all of its predicted proteins is in the basic range, and with an overepresentation of lysine. Enzymatically active plasmin can be generated on the Borrelia surface by exogenous urokinase, and remains protected from inhibition with a− 2 anti-plasmin. The spirochetes are not harmed by this borrowed surface plasmin, and in vitro studies have shown increased invasiveness by plasmin-bound Borrelia.

JS-16

Investigating the Pathogenesis of Granulocytic Ehrlichiosis using Neutrophils and Myeloid Cell Lines. J. STEPHEN DUMLER. Div. Medical Microbiology, Dept. Pathology, The Johns Hopkins University School of Medicine, Baltimore MD. Email: SDUMLER@JHMI.EDU

Ehrlichiae are obligate intracellular bacteria that reside in endosomes of infected cells in mammalian and tick hosts. Despite a strong mammalian host cell tropism that exists with certain *Ehrlichia* species, the dual niches of these bacteria imply adaptations specific for both vertebrate and invertebrate cells. In some mammalian hosts, infection leads to significant pathologic processes, a high degree of morbidity, and a low level of mortality. In order to model infection and to study isolated pathogenetic processes in mammalian hosts, the infectious agent can be propagated in undifferentiated and differentiated myeloid cell lines such as HL-60 cells, and in mature primary neutrophils. Using these systems, we have examined potential host cell ligands and bacterial adhesins, functional perturbations of infected host cells, and non-adhesin bacterial gene products that interact with infected host cells and potentially influence host cell function. The unique neutrophil niche of this bacterium provides important opportunities to better understand how ehrlichiae manipulate the host cell for propagation and affect host cell function to cause disease.

JS-17

Tick Cell Culture: New Approaches for *Anaplasma* Research. K.M. KOCAN[1], E.F. Blouin[1], A. F. Barbet[2], J.T. Saliki[1], B.R. McEwen[1], P.F.M. Meeus[2].[1]College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078, and [2]Dept. Pathobiology, University of Florida, Gainesville, FL 32611. Email: KMK285@okstate.edu

Recently, the ehrlichial cattle pathogen, *Anaplasma marginale*, was propagated in a continuous tick cell line (IDE8) that was derived from embryos of Ixodes scapularis. Prior to the development of the culture system, *A. marginale* research relied on use of infected cattle or ticks as an antigen source. Cultivation of *A. marginale* has provided new research approaches and applications. Development of *A. marginale* in IDE8 cells was easily documented and found to be similar to that described in naturally infected ticks. The culture system, therefore, can be used as a model for studying tick/pathogen interactions. The six major surface proteins described on bovine erythrocyte-derived *A. marginale* were present on the culture-derived organisms and, therefore, the cultures show promise as a source of antigen for vaccines and serodiagnosis. The culture system is being used to study the development of antigenic variation of *A. marginale*. Clones of *A. marginale* have been developed from single infected IDE8 cells selected with a micropipette. These clones are being used to document antigenic variation of *A. marginale* by comparing the genomic sequences of epitopes from multigene families after passage in culture, cattle and ticks. The *A. marginale* cell culture system was used to develop a drug testing assay and an in vitro neutralization assay for testing the role that polyclonal and monoclonal antibodies play in pathogen invasion. *A. marginale* was the first pathogen cultivated in the IDE8 cells, but subsequently *Ehrlichia canis, E. chaffeensis*, the human granulocytic ehrlichial agent (HGE) and *Cowdria ruminantium* were propagated in the IDE8 cells. The cell culture system may also provide new research opportunities for these important pathogens.

JS-18

Lyme disease spirochetes at the tick and mammalian host interface: physiological and immunological consequences. M. OBONYO,[1] U.G. Munderloh,[2] V. Fingerle, [3] B. Wilske,[3] and T.J. Kurtti.[2] Department of Medicine, University of California, La Jolla, CA,[1] Department of Entomology, University of Minnesota, St. Paul, MN,[2] and Max von Pettenkofer-Institut fur Hygiene und Medizinische Mikrobiologie, Ludwig-Maximilians-Universitat Munchen, Munich, Germany,[3] E-mail: mobonyo@ucsd.edu

Lyme disease, caused by the spirochete *Borrelia burgdorferi*, is the most commonly reported arthropod-borne infection in the USA. The spirochete is transmitted to the mammalian host by ticks belonging to the *Ixodes* genus. Despite much research on outer surface proteins of *Borrelia burgdorferi*, little is known about the regulation mechanisms of these proteins and their function in bacterial pathogenesis. *Borrelia burgdorferi* sensu stricto downregulates outer surface protein A (OspA) and upregulates outer surface protein C (OspC) during tick feeding. The switching of these proteins correlates with increased spirochetal infectivity for the mammal. We examined the expression of OspA and OspC during in vitro cultivation. The role of tick factors in modulating expression of OspA and OspC was studied by cocultivating spirochetes with vector tick cells. To determine if OspC plays a role in infectivity of *Borrelia burgdorferi*, hamsters were inoculated with spirochetes expressing high or undetectable amounts of OspC. Spirochetes cultured axenically expressed OspC at 34°C and 37°C but OspC was undetectable in spirochetes cultured at 31°C or lower. In contrast, OspA was expressed at all temperatures used in our study. Cocultivation of spirochetes with tick cells at 37°C resulted in an enhanced production of OspC. In contrast, the amount of OspA expressed was highest at 31°C and declined with increasing temperature and was lowest at 37°C. Infectivity studies indicated that there is a strong correlation between OspC expression in *Borrelia burgdorferi* and hamster infection. All hamsters inoculated with OspC expressing spirochetes became infected. All the infected hamsters had an antibody response to OspC. In contrast, all non-infected hamsters lacked antibodies to OspC. Using the tick cell culture system and temperature, we demonstrate differential expression of OspA and OspC. We also show that spirochetes cultured at 37°C had an enhanced infectivity for hamsters, suggesting a strong correlation between OspC expression and infectivity.

J-19

Bioinformatics in the New Millennium. Darrell O. Ricke, PhD. Novartis Agricultural Discovery Institute, Inc., 3115 Merryfield Row, Suite 100, San Diego, CA 92121. Email: darrell.ricke@nadii.novartis.com, The new millennium starts with the pace of biological discovery accelerating at an astounding rate. As genetics has progressed from studies of individual genes to entire genomes, complementary approaches are rapidly progressing. These approaches are described as Genomics, Functional Genomics, Proteomics, and Structural Genomics. This new wealth of scientific information presents bioinformatics with the challenge of turning data into knowledge and biological understanding. This requires solutions that include data integration, data warehouses, distributive computing, enterprise solutions, statistics, software engineering and informatics. Integrated solutions will be facilitated by object oriented component technologies working with data warehouses. Much can be learned from open system approaches, best illustrated by the success and quality of the Linux Operating System. Open architecture solutions will greatly facilitate the development of large integrated solutions. Ideal systems will facilitate the navigation of: mapped loci, genes, promoters, gene expression profiles, protein expression information, sequence motifs, protein structure information, protein function classification, protein function assays, pathway information, taxonomic relationship of species, phenotypic information, pedigree information and evolutionary relationships with other sequences. Approaches that model a cell or a living organism will also facilitate learning about biology and the life that surrounds us.

JS-20

Informatics for High-Throughput Functional Genomics. CRAIG LIDDELL. Paradigm Genetics Inc., Research Triangle Park, NC 27709. Email cliddell@paragen.com

Multivariate statistical procedures have been developed for many scientific applications. The advent of biotechnology with multiple heterogeneous data sets, derived from high throughput functional analyses, provides a challenge and new applications for these statistical procedures. Mature laboratory information management systems are required for data collection and specimen tracking. These systems must be efficient, robust and require quality control and normalization procedures that allow partitioning of systematic error. Qualitative discriminate (assumption) analysis or data transformation methods can be used to cluster data efficiently in a high throughput setting to prepare data for advanced analyses. Complex, normalized, multi-factor relationships, where there are no dependent or independent variables, can be analyzed using these multivariate approaches. This application also allows high order relationships to be explored between multiple data types. The use of multivariate statistics in bioinformatics allows predictions to be generated about complex genotype-phenotype relationships and biochemical pathway regulation.

JS-21

Gene to Screen: Genomics, Screening, and Information Management. R. KOCHARR. Sphinx Pharmaceuticals, 20 TW Alexander Dr., Research Triangle Park, NC 27709. E-mail: rkochhar@lilly.com

The visible early potential of the marriage of Biology, Chemistry, and Technology, utilizing the rapid advances in each, foreshadows great power in streamlining interplay of the disciplines. The resultant effort yields more of a quest than a product: constant optimization of the integration while managing and effectively utilizing the gargantuan volumes of data produced through new Biology and Chemistry methodologies. Collaborative effort in search of improved integration and information management is key for current success as well as for creating the foundation to leverage the evolution of science and technology.

JS-22

In Vitro Propagation for Ex Situ Preservation of Endangered Plant Species V.C. PENCE and J.R. Clark. Center for Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220. E-mail: vcpence@aol.com

In a collaboration with the Center for Plant Conservation and its participating institutions, CREW is applying in vitro techniques to over 20 difficult-to-propagate endangered species from the mainland U.S. These species include herbaceous and woody species, monocots, dicots, and species from wet and dry environments. They represent a variety of barriers to propagation. Some produce little or no viable seed; some have dormant seeds or slow germination rates; and some have highly specialized requirements for germination, as with parasitic plants and orchids. The variety of approaches taken for the specific needs of these endangered taxa draws, in turn, upon the variety of in vitro techniques which are available. When seeds are few, shoot cultures have been initiated from off-shoots or seedlings and used to increase plant numbers. When seeds are not available, in vitro collection has been used to initiate shoot cultures. When seed germination is difficult, in vitro germination or embryo culture has been applied, in order to initiate shoot cultures. Standard in vitro germination techniques have been applied to orchids, followed by clonal propagation in vitro; while the in vitro germination of parasitic species with very specialized germination requirements is being investigated. As many genetic lines as possible of each species are established and are maintained and propagated separately. Plants produced in vitro are acclimated to soil and then returned to collaborators for research, ex situ growth, or reintroduction. The tissue culture lines of these species are cryopreserved for long-term germplasm storage at CREW. Research supported in part by Institute of Museum and Library Services grants Nos. IC-50056-95 and IC-70248-97.

JS-24

The Role of the Frozen Zoo in Genetic Research for the Preservation of Endangered Species. M.L. HOUCK and A.T. Kumamoto. Center for Reproduction of Endangered Species, Zoological Society of San Diego, San Diego, CA 92112. E-mail: mhouck@sandiego.org

Since 1975 the Zoological Society of San Diego has supported the establishment and maintenance of a frozen cell repository of viable fibroblast cell lines from endangered and other threatened species. Improvements in primary culture initiation techniques and media development have made it possible to optimize growth conditions of cell lines from a wide variety of taxa. The Frozen Zoo collection currently contains somatic cell lines from more than 4,200 individuals providing access to the genomes of 370 species and subspecies representing 16 mammalian and six avian orders. Approximately one third of these species are listed in the IUCN Red List of Threatened Animals. Samples are acquired from zoological institutions worldwide as well as from field researchers studying animals in the wild. Field techniques that allow sample collection from remote locations have increased the diversity of populations sampled. This collection, utilized by local, national and international investigators, provides a unique resource for comparative genetic studies at the chromosomal and molecular levels that have direct application to the conservation management of endangered species. The broad scope of genetic research supported by living cell collections such as the Frozen Zoo will be addressed.

JS-25

Development of in vitro oocyte maturation and cryopreservation techniques for the domestic dog as a model for germplasm rescue in endangered carnivores. B.S. DURRANT. Center for Reproduction of Endangered Species, San Diego, CA, 92112. bdurrant@ucsd.edu

Preservation of maximum genetic diversity in endangered species requires the routine post-mortem rescue of germplasm. Epididymal sperm extraction is an effective technique for preservation of the male gamete. However, unlike epididymal sperm, ovarian ova require precise in vitro maturation culture conditions prior to fertilization. The domestic dog served as a model for oocyte in vitro maturation and cryopreservation studies. Following enzymatic digestion of ovary tissue, isolated follicular oocytes were cultured individually in plastic 96-well plates for 66 hr in synthetic medium supplemented with EGF, E2, LH and/or FSH. Oocytes were then fixed, stained, and examined for nuclear progression to MI-MII. EGF alone did not increase maturation rates over unsupplemented controls, but significantly enhanced progression to MI-MII in the presence of LH and FSH. The addition of E2 decreased oocyte maturation regardless of the presence or absence of hormone supplements. Preliminary cryopreservation studies compared integrity of the germinal vesicle and morphology of fresh oocytes, those frozen within ovary tissue, and those isolated before freezing. Isolated oocytes survived slow freezing with 95% normal morphology (not different from fresh oocytes), but only 50% of oocytes frozen in ovary tissue were classified as normal after thawing. Fifty-eight percent of frozen isolated oocytes retained intact germinal vesicles (compared to 82% in fresh oocytes), while a mere 18% of oocytes isolated after freezing in ovary tissue had maturation potential.

JS-26

Pantropic Retroviral Vectors for Gene Expression in Fish Cell Lines: Toward a Rational Approach to Genetic Engineering of Disease Resistance in Fish. J.C. BURNS[1], S. Bakalis[1], V. Boulo[2]. [1]Dept. of Pediatrics, UCSD School of Medicine, La Jolla, CA 92093-0830; [2]DRIM, IFREMER-CNRS, Univ. Montpellier II, 34095 Montpellier, France. Email: jcburns@ucsd.edu

Pantropic retroviral vectors contain the envelope glycoprotein of the vesicular stomatitis virus (VSV-G) and mediate vector attachment to a wide variety of cells. Transfer and expression of foreign genes has been achieved in cell lines derived from zebrafish (ZF4), rainbow trout (RTG-2), chinook salmon (CHSE-214), and bluegill (BF-2). To aid in the design of vectors for transgene expression in fish cell lines, we compared promoters using the firefly luciferase reporter gene. Cell lines were infected with vectors expressing luciferase from the following promoters: Moloney murine leukemia virus (MoMLV) long terminal repeat (LTR), Rous sarcoma virus (RSV) LTR, and human cytomegalovirus immediate early promoter (CMV). Cell lysates were prepared 72 h after infection and luciferase assays were performed. The retroviral LTRs mediated the highest levels of luciferase expression. Based on these data, vectors were created with the gene of interest cloned downstream from the MoMLV LTR. To explore whether expression of the human, interferon-inducible GTPase, MxA, could protect fish cells against challenge with an RNA virus, we created the vector L-MxA-RNL, in which the MoMLV LTR drives expression of the human MxA cDNA and the RSV LTR drives expression of the selectable marker, neomycin phosphotransferase. This vector was used to infect RTG-2 cells and G418-resistant clones were selected. Challenge of clones with the rhabdovirus, infectious hematopoietic necrosis virus (IHNV), will test the antiviral properties of human MxA expressed in fish cells. These types of *in vitro* experiments will permit a rationale approach to the genetic engineering of disease resistance in fish species of importance to the aquaculture industry.

JS-27

Fish Embryo Cell Cultures for Cell-Mediated Gene Transfer. P. COLLODI, C. Ma, and L. Fan. Dept. of Animal Sciences, Purdue University, West Lafayette, IN 47907. Email: pcollodi@ansc.purdue.edu

We are working to establish a pluripotent embryonal stem cell line from zebrafish for studies of cell differentiation and production of transgenic and knockout mutant lines of fish. Cell cultures derived from zebrafish blastula-stage embryos exhibit in vitro characteristics of pluripotency including an ES-like cell morphology, alkaline phosphatase activity and the ability to differentiate into the multiple cell types in culture. Short-term embryo cell cultures maintained on a feeder layer of stromal cells derived from trout spleen, possess the capacity to generate germ-line chimeras when introduced into host blastulas. Contribution to the host germ line was determined by the inheritance in the F1 generation of a marker gene and pigmentation donated by the cultured cells. Work is being conducted to extend the length of time that the cells can be cultured without losing the capacity to contribute to the host germ line. In addition to the production of transgenic fish, we are utilizing the embryo cell cultures to study the influence of extracellular matrix proteins on cell differentiation. From this work we have identified a novel from of fibronectin (ZFN) that is expressed in zebrafish embryos. ZFN exhibits a truncated structure, possessing 9 type I, 2 type II and only three type III repeats along with a unique C-terminal tail consisting of 20 amino acids. Work is in progress to determine the function of ZFN during zebrafish development.

JS-28

In Vitro Cultivation and Characterization of Viruses of Marine Organisms. C. BUCK[1], D. Barnes[1], R. Davis and M. Walsh[2]. [1]American Type Culture Collection, Manassas, VA and [2]Sea World of Florida, Orlando FL. E-mail: cbuck@atcc.org

Despite advances in molecular biology that have in the case of hepatitis B allowed the development of a vaccine and screening system, without a suitable experimental host, the isolation and growth of a virus remains central to the study of a virus. The ability to grow the virus in vitro is in turn intimately linked to the availability of suitable host cells. During the past 10 years several efforts to isolate virus from marine vertebrates illustrate this interdependence. In the first instance isolation of virus was attempted from necropsy samples taken from a captive mature killer whale that died 48hr after presenting with reduced appetite and lethargy. Samples from brain, lung and kidney were screened on African green monkey kidney (AGMK), bovine turbinate, and SP-1K (dolphin) cell cultures. In the face of bacterial contamination, CPE was only apparent in the SP-1K culture. This isolation lead to the identification of St. Louis encephalitis virus in the necropsy samples. In a second instance, the lack of a suitable host cell has prevented the growth of a poxvirus from tissue where it has been visualized by EM. In a third instance, shifting an isolate to A-72 cells revealed the presence of a second previously unrecognized viral co-isolate. In the most recent example, an effort was made to isolate the etiologic agent responsible for the development of transmissible, granulomas on captive green moray eels (*Gymnothorax funebris*). Lacking an eel line to use as a host for screening, a diseased animal was sacrificed and several tissue samples taken for development of cell culture along with samples to freeze for subsequent virus isolation attempts. It was subsequently noted that the only cells from the eel to grow well were from the granuloma cells and subsequent tests revealed that those cultured cells, as well as cell-free culture supernatant from the cells, were positive for reverse transcriptase activity. Subsequent tests of eel tumor cell RNA with universal retrovirus PCR primers demonstrated the presence of host nucleic acid able to serve as template for a retrovirus-like amplicon. Efforts are ongoing to characterize this putative retrovirus.

JS-29

Algal Phycocyanins Promote Growth of Animal Cells in Culture Kazuki Shinohara. National Food Research Institute, MAFF, Tsukuba, Ibaraki, 305-8642 Japan E-mail: kazuki@nfri.affrc.go.jp

To culture animal cells including fish-derived cells in serum-free medium, it is necessary to add the growth factors or hormones which the cells require. Many growth factors have been already found, such as isulin, transferrin, ethanolamine, selelnite and so on. These factors are of animal origins. Few growth factors have been isolated from plant origins. It is expected that the substances affecting the growth of animal cells may exist in plants because fresh-water fish are known to take microalgae. For detection of growth factors, plant which are obtained in large amounts with the equal quality whenever require are desirable. Microalgae are easy to culture, highly productive, and easily reproduced. Among the microalgae, thermophilic algae are noteworthy because their constituents are expected to be stable. The detection of growth factors for animal cells were then performed from the extract of a thermophilic blue-green algae, qi rSynecchoccus elongatus var. (Syne. elongatus var.) q/i r. Consequently, it was found that the non-dialyzable extract of qi rSyne. elongatus var q/i r. promotes the growth of RPMI8226 myeloma, Molt-4 acute T-lymphocytic leukemia, HMY-2 and HO-323 B-lymphoblastoid cell lines in their serum-free media. The growth-promoting principles in qi rSyne. elongatus var q/i r. were found to be biliprotein of phycocyanin and allophycocyanin. Both biliprotein promoted the growth of RPMI8226 cells; however, allophycocyanin was more active than phyocyanin.

JS-30

FGF Inhibits Neurogenesis in Primary Cultures of Early Zebrafish Embryo Cells. D.W. BARNES, N.N. Singh, K. Fischer and O. Hedstrom. American Type Culture Collection, Division of Cell, Developmental and Molecular Biology, Manassas VA 20110 E-mail: barnes@atcc.org

Toward the goal of in vitro culture of zebrafish embryonic stem cells and an understanding of extracellular influences on early embryonic development, we examined basic FGF regulation of developmental markers in cell cultures derived from early zebrafish embryos. Examined by reverse transcription PCR were stem/primordial germ cell markers pou-2 and vas, neural markers zp-50, pax{zf-a}, en-3 and wnt-1, and mesodermal markers gsc and myoD. Previously we showed that FGF prevents the development of zebrafish neural crest-derived pigment cells in vitro. In our culture system, FGF reduced expression of neural-specific markers, implicating the FGF family in supression of early neural cell development. Exposure to FGF for 24 hours was sufficient to suppress neural marker expression for a subsequent four days of culture, while absence of FGF for the first 24 hours of culture nullified the effect of FGF added subsequently. FGF increased expresssion of gsc and myoD in a reversible manner. Vas expression was unaffected, while pou-2 expression decreased with time in culture in the presence or absence of FGF. However, in situ hybridization identified a subpopulation of cells expressing pou-2, suggesting the possible continued existence of undifferentiated stem cells in the cultures.

JS-31

Prospective acute phase proteins expressed in hepatocytes of Rainbow trout. C.J. BAYNE, L.G.Gerwick, F. Kazuhiro, M. Nakao and T. Yano. Department of Zoology, Oregon State University, Corvallis and Department of Fisheries, Kyushu University, Fukuoka. E-mail:baynec@bcc.orst.edu

A resurgence of interest in the evolution of innate immune responses in both vertebrates and invertebrates has encouraged us to develop the Rainbow trout as a desirable model. Immuno-competence is capable of upregulation in both innate and adaptive immune systems. Our starting point for developing the model has been to profile the hepatic acute phase response to inflammatory stimuli. Both proteomic and molecular genetic approaches have been evaluated. A homolog of the human 'precerebellin' protein has been identified using 2-dimensional gel electrophoresis to isolate the protein from fish plasma, followed by amino acid sequencing of tryptic peptides and PCR using degenerate primers. The full length sequence of the cDNA has been obtained. The molecular genetic approach took advantage of subtraction suppressive hybridization between cDNAs from the livers of trout injected with (i) an inflammatory stimulus and (ii) nothing. This has yielded a larger number of putative homologs. As a result of obtaining sequences for several independently cloned fragments of the 'differentially regulated trout protein', a consensus sequence is available. About 50 additional cDNAs (of 200 sequences obtained) are significantly similar to known proteins. Of these, 11 sequences have been reported previously from fish species. Following completion of sequencing of another 200 clones, efforts will be directed towards obtaining full length sequences for selected cDNAs.

JS-32

Establishment of an Abalone Digestive Gland Cell Line Secreting Various Glycosidases in Protein-free Culture. SANETAKA SHIRAHATA[1], Kenichi Kusumoto[1] and Yuto Kamei[2]. [1]Graduate School of Genetic Resources Technology, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan; [2]Marine & Highland Bioscience Center, Saga University, 152-1 Shonan-cho, Karatsu, Saga 847-0021, Japan. Mail: SIRAHATA@GRT.KYUSHU-U.AC.JP.

Establishment of digestion gland cell lines derived from sea mollusks will be useful to produce various glycosidases digesting polysaccharides of seaweed as well as to clarify the cell physiology of sea mollusks. A cell line designated as ADG was established from an abalone digestive gland using ERDF medium supplemented with 8% fetal bovine serum (FBS), 8% abalone hemolymph, and high concentrations of NaCl, KCl, $MgCl_2$, $MgSO_4$ and $CaCl_2$. ADG cells proliferated better in protein-free medium than in FBS-supplemented medium. Among 9 kinds of media examined, ERDF was optimal for cell growth. ADG cells secreted 13 different kinds of glycosidase in protein-free medium. When ADG cells were cultured in Grace's insect cell medium, the activity of some secreted glycosidases increased 25-fold to 65-fold per cell as compared with control cells cultured in ERDF medium. The electron microscopic analysis revealed that an ADG cell contained several nuclei, suggesting that it may be derived from protist cells. Alpha-D-Galactosidase hydrolyzing p-nitrophenyl galctopyranoside was purified 130-fold from the spent medium of ADG cells. The enzyme was suggested to consist of single polypeptide chain of 43 or 42 kDa by SDS polyacrylamide gel electrophoresis or gel filtration analysis, respectively. The activity of the enzyme was optimal at pH 5.5 and 37 ¡C. and the enzyme was most stable at pH 5.5.

JS-33

The Manzanar Mangrove Initiative: Low-Tech Biotech to Plant Mangroves Where They Do Not Occur in Nature. GORDON SATO, Simon Tecleab, Million Ghirmay, Tesfom Ghezae, Samuel Negassi, and Robert Riley. Ministry of Fisheries, Massawa, ERITREA. Email imu@eol.com.er

The Manzanar Project is a venture to eliminate world famine and poverty in underdeveloped countries such as Somalia and Eritrea (Ethiopia). This effort is to convert coastal deserts into rich agricultural fields through the culture of microscopic algae in seawater ponds and the cultivation of mangrove timber crop species with a seawater irrigation system. Only 15% of the intertidal zone of Eritrea contain mangroves. Mangroves occur in mersas where the seasonal rains collect land nutrients and flow into the sea. In a comparison with complete algae media, seawater is low in nitrogen, phosphorus and iron concentration. Mangrove tree, irrigated with sea water supplemented with DAP and iron, have been successfully established in areas of dead rocky coral reefs, anoxic soil mud flats and low lying tidal beds of the Massawa shrimp farms. The Manzanar Mangrove Initiative is cost effective to provide timber crops. This initiative serves as a model to produce other ornamental and food crops and provides supplements to enrich the sea for fishes.

JS-35

Neural Stem and Progenitor Cells in the Mammalian Brain. DEREK VAN DER KOOY, Department of Anatomy and Cell Biology, University of Toronto,Toronto,ON, Canada M5S 1A8. E-mail: derek.van.der.kooy@utoronto.ca

Brain precursor cells exist in two flavours: neural stem cells (multipotential with extensive capacity for self-renewal) and neural progenitor cells (more restricted in potential with less capacity for self-renewal). Both cell types have now been characterized in vitro from the embryonic as well as adult mammalian brain. Moreover, infusion of growth factors into the adult mammalian brain in vivo can stimulate these precursor cells to produce new neurons and glial cells.

JS-37

The Structures and Cytolytic Mechanism of *Bacillus thuringiensis* Insecticidal Toxins. DAVID J. ELLAR, Biochemistry Department, Cambridge University, Tennis Court Road, Cambridge, CB2 1GA, UK Email: dje1@mole.bio.cam.ac.uk

The spore-forming bacterium *Bacillus thuringiensis* produces highly specific insecticidal proteins (delta-endotoxins) grouped into two main families—Cry and Cyt toxins—by amino acid sequence similarity. Both types of toxin bind to insect-specific receptors on the surface of gut epithelial cells and in a second irreversible step insert into the cell membrane to form leakage channels that result in cell death by colloid osmotic lysis. Studies with insect cell cultures have helped to elucidate the toxin mode of action. The protein nature of these toxins coupled with genetic engineering offers great potential for pesticide improvement and resistance management and has allowed them to be expressed in plants as systemic biopesticides. The X-ray structure of the first Cry toxin revealed putative membrane insertion and receptor binding domains whose functions are being explored by intensive mutagenesis and domain swapping. The first Cyt toxin structure has now been described and is entirely different from the Cry toxins—despite their similar toxic mechanism. Current biochemical and genetic attempts to define structure-activity relationships for these toxins will be reviewed. The recent cloning and sequencing of Cry toxin receptors have further enhanced the potential of these pesticides. These receptors are transmembrane proteins exposed on the lumen surface of midgut epithelial cells. The structure of these receptors, their role in toxin recognition, membrane pore formation and toxin resistance will be discussed. Although the best *Bacillus thuringiensis* toxins are insecticidal at concentrations typical of potent chemical pesticides, many of them are ten or even 1,000 times less potent. These less potent ones include some which are potentially the most important from the standpoint of crop protection and disease vector control. Molecular strategies to enhance toxin potency will be discussed.

JS-38

Expression of *Bt* Toxin Genes in Plants: Implications for Transgene Design. SCOTT H. DIEHN and Pamela J. Green. MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312. Email: diehnsh@phibred.com and green@msu.edu.

Bt toxins are a family of bacterial proteins with specific insecticidal activity. Unfortunately, the mRNA of *Bt* toxin genes fails to reach levels in transgenic plants that are necessary for protection against insect pests. Synthetic genes have been used to circumvent this problem; however, the reason why the wild-type mRNA fails to accumulate was not well understood until recently. We have found that sequences in the coding region of a *cry1Ac Bt* toxin gene mediate the rapid turnover and premature polyadenylation of the mRNA in plant cells. Further experiments analyzing the poly(A) sites in the *cry1Ac* coding region and the poly(A) sites from several plant genes indicate that poly(A) signal utilization in tobacco is more stringent than in maize. This difference likely represents a dicot/monocot difference that is relevant to transgene design.

JS-39

The Diversity of Bt-Transgenic Crops. W.J. MOAR. Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849. E-mail: MOAR@MINDSPRING.COM

The use of plants expressing insecticidal proteins from *Bacillus thuringiensis* (Bt) have escalated recently due to the demand for alternatives to traditional insecticides for insect control and the tremendous progress scientists have made in plant transformation and regeneration. Although about 25 million acres of Bt transgenic plants were grown in the US alone in 1999, this acreage was represented almost exclusively by only 3 crops (cotton, corn, and potatoes) and only 4 Bt proteins targeted at primarily only 5–6 insect species. Although the number of Bt proteins expressed in plants is not expected to drastically increase in the near future, the number of crop plants and associated cultivars is expected to drastically increase. This presentation will discuss Bt transgenic crop cultivars and associated Bt toxins currently available commercially and new crop plants and associated Bt toxins expected on the market within the next several years.

JS-40

Issues Affecting Corn Growers' Decisions to Plant (or Not Plant) Bt Corn. K. L. STEFFEY. Department of Crop Sciences, University of Illinois, Urbana, IL 61801. E-mail: ksteffey@uiuc.edu

Bt corn offers corn growers a viable alternative for managing the European corn borer, *Ostrinia nubilalis*, one of the most important insect pests of corn in the United States. Since its commercialization in 1996, Bt corn has been planted on an increasing percentage of acres. Some experts estimate that about 30% of the corn acreage in the United States was planted to Bt corn in 1999. For the most part, Bt corn is very effective for controlling European corn borers, as well as southwestern corn borers, *Diatraea grandiosella*, a serious pest in the western Corn Belt. In addition, Bt corn offers some level of protection against corn earworms (*Helicoverpa zea*), fall armyworms (*Spodoptera frugiperda*), and stalk borers (*Papaipema nebris*). In years when densities of European and/or southwestern corn borers exceed economic injury levels, corn growers receive a positive return on their investment for Bt corn. Economic returns can be substantial when densities of the pests are high, yield expectations are high, and the market value of corn is high. However, in years when densities of the pests are low, yields are low, and the market value of corn is low (prevailing circumstances in 1999), corn growers lose money on their investment in Bt corn. Unfortunately, predicting corn borer densities, yields, and market prices is difficult. Therefore, corn growers must base their decision to plant or not plant Bt corn primarily on the frequency of economic infestations of corn borers in their area over time. After 2 years (1998 and 1999) of low densities of European corn borers, corn growers are wondering whether they need Bt corn. Complicating growers' decisions to plant or not plant Bt corn in 2000 are the controversial issues addressed extensively in the media throughout 1999. The potential for development of resistance to Bt in populations of target pests concerned scientists long before Bt corn was commercialized. In response to this concern, university, government, and industry scientists worked together to develop viable resistance management strategies to ensure the effectiveness of the technology well into the future. In 1999, at the urging of the National Corn Growers Association, the scientists reached a consensus regarding the best resistance management plan, which included a minimum of 20% of a grower's corn acres devoted to non-Bt corn refuges near or within Bt cornfields. Many other issues surfaced in 1999, and these issues have had a profound effect on growers' considerations for planting Bt corn in 2000. Among the most widely discussed and hotly debated issues regarding GMOs (genetically modified organisms) are potential effects on nontarget organisms (especially monarch butterfly caterpillars, *Danaus plexippus*); concerns about the safety of foods processed from GMOs; and potential environmental and ecological impacts of GMO crops. These issues have resulted in GMO grain import limitations, demands for segregating GMO grains from non-GMO grains, and the potential labeling of foods processed from GMOs. As these issues are debated in the popular press, scientists continue to investigate both the benefits and risks associated with all transgenic crops, including Bt corn. Results from research efforts will continue to help us reconfigure strategies for use and management of transgenic crops.

JS-41

Effects of Bt-transgenic Crops on Nontarget Organisms. A. HILBECK. Swiss Federal Institute of Technology, Geobotanical Institute, CH-8044 Zurich, Switzerland. E-mail: hilbeck@geobot.umnw.ethz.ch

The development of genetically engineered plants proceeds at a high speed with adoption rates promising enormous profits. While currently 'agronomic' traits (herbicide resistance and insect resistance) dominate, traits conferring 'quality' traits (altered oil composition, etc.) will begin to take over soon. However, economically the most promising future lies in the development and marketing of crop plants expressing pharmaceutical or nutraceutical ('functional foods') traits. Therefore, future agricultural but, eventually, also natural ecosystems will be challenged by large-scale introductions of entirely novel genes and gene products in entirely novel combinations at high frequencies all of which will have unknown impacts on the biodiversity of the associated complex of 'nontarget' organisms of these plants. In a multiple year research project, we investigated the tritrophic effects of transgenic Bt-expressing corn on a number of predator species. We found prey-mediated effects of transgenic Bt-corn causing significantly higher mortality of an important insect natural enemy, the Green Lacewing. This was the first report on the susceptibility of a third trophic level organisms to a Bt-toxin which was believed to only affect herbivores. In further lab trials, we confirmed that the route of exposure (prey-mediated or fed directly) and the origin of the Bt (from transgenic or incorporated diet) strongly influenced the degree of mortality. We further confirmed direct toxicity of Cry1Ab toxin by observing receptor binding in lacewing larvae midgut epithelium. Field implications will be difficult to assess because they are likely to result in long-term chronic effects interfering in very intricate ways with complex ecosystem processes. Therefore, areawide monitoring programs need to be established to detect possible adverse effects on ecosystem functioning and biodiversity at an as early stage as possible.

JS-42

Bt Resistance: Got Refuge? BRUCE E. TABASHNIK,Timothy J. Dennehy, Yves Carrière, Yong-Biao Liu, Susan K. Meyer, Amanda L. Patin, and Maria A. Sims Department of Entomology, University of Arizona, Tucson, AZ 85721 E-mail: brucet@ag.arizona.edu

Many insects have evolved resistance to Bacillus thuringiensis (Bt) toxins in the laboratory and diamondback moth has evolved resistance to sprays of Bt toxins in the field. The refuge strategy is the major tactic used for slowing evolution of insect resistance to crops that are genetically modified to produce Bt toxins. Refuges consist of host plants that do not produce Bt toxins and thus allow survival of susceptible insects. So far, Bt cotton has been extremely effective in controlling the pink bollworm (Pectinophora gossypiella), a global pest of cotton that is the key lepidopteran pest of cotton in Arizona. However, intense selection for pink bollworm resistance to Bt cotton is likely because of the high proportion of Bt cotton in Arizona and limited use of alternate host plants by this pest. The refuge strategy is expected to be most effective when three assumptions are valid: 1) random mating occurs between susceptible adults emerging from non-Bt cotton refuges and resistant adults emerging from Bt cotton, 2) inheritance of resistance is recessive, and 3) the frequency of alleles conferring resistance is less than 0.001. We are testing these assumptions for pink bollworm in Arizona with experiments in the field, greenhouse, and laboratory. Results to date suggest that violations of one or more of the assumptions are likely. Thus, pink bollworm is at high risk for rapid evolution of resistance to Bt cotton.

JS-43

New Insecticidal Proteins for Use in Transgenic Crops. BRIAN A. FEDERICI. S Department of Entomology & Interdepartmental Graduate Program in Genetics University of California, Riverside, Riverside, California 92521. E-mail: brian.federici@ucr.edu

Numerous crop plants have been engineered to produce insecticidal proteins using conventional transformation technologies. Most insecticidal proteins engineered into crop plants to date are Cry proteins originating from the bacterium, Bacillus *thuringiensis*(Bt), and all crops currently in production produce only a single Cry protein. Concern has been raised about this insect control strategy in that it presents the possibility for the development of resistance in the target populations. These concerns are justified based on the rapid development of resistance and cross-resistance to single Cry proteins in the laboratory when insects are placed under strong selection pressure. Resistance management studies with bacterial insecticides have shown that resistance can be delayed by using mixtures of insecticidal proteins and proteins with different modes of action. For example, the Bt Cyt1A protein, which attacks the lipid portion of the midgut microvillar membrane, delays the development of resistance to Cry proteins when used in combination with one or more of these proteins. Combination of multiple Cry proteins with a single Cyt protein can delay the development of resistance for 50 sequential generations, even when insects are selected with toxin concentrations that kill 90% of the insects in each successive generation. The principles of using multiple toxins and combining toxins with different modes of action offer considerable promise for managing resistance to Bt-crops based on Cry proteins. Among the proteins currently being evaluated for use in Bt-crops to manage resistance are several Bt proteins produced during the vegetative stage of growth, and insecticidal proteins from non-Bt sources including those from symbiotic bacteria associated with insect-parasitic nematodes. Though years away from commercial use, these new insecticidal proteins offer high potential for managing insect resistance to insecticidal transgenic crops.

JS-44

Suppression of Cancer and Diabetes by Anti-oxidizing Water. SANETAKA SHIRAHATA. Graduate school of Genetic Resources Technology, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan. Email: SIRAHATA@GRT.KYUSHU-U.AC.JP.

It has long been established that reactive oxygen species (ROS) caused numerous damages to biomolecules and cellular structures that resulted in the development of a variety of pathologic states such as cancer, diabetes, and aging. Electrolyzed-reduced water (ERW) which is produced near cathode by electrolysis was demonstrated to be anti-oxidizing water that contained high concentration of hydrogen and could scavenge ROS (Shirahata et al., 1997). Recently we found that Hita natural reduced water (HNRW) drawn from the underground of Hita city (Japan) was also anti-oxidizing water. ERW reversibly caused passage number-dependent telomere shortening in human lung cancer A549 and human uterine cancer HeLa cells without change of telomerase activity. From the analysis of telomere binding proteins, it was suggested that ERW inhibited the binding of telomerase to telomere by decreasing the binding activities of telomere binding proteins. Both ERW and HNRW suppressed the growth of cancer cells transplanted into mice, demonstrating their anticancer effects in vivo. ROS is known to cause reduction of glucose uptake by inhibiting the insulin-signaling pathway. ERW scavenged intracellular ROS and stimulated glucose uptake without insulin in both rat L6 myotubes and mouse 3T3/L1 adipocytes. This insulin-like activity of ERW was mediated by the activation of PI-3 kinase. HNRW also exhibited insulin-like effect on sugar uptake, suggesting that ERW and HNRW may improve insulin-independent diabetes mellitus.

I-1

Foundations for Achievement in the New Millennium. JAMES L. VAUGHN. 5679 Vantage Point Road, Columbia, MD 21044. E-mail: jvaughn567@aol.com.

At the First International Colloquium on Invertebrate Tissue Culture in 1962 Tom Grace reported the two-year growth of insect cells in culture. The establishment of these cell lines culminated 47 years of research by scientists in this field and provided a method for developing cell lines and a basic medium formulation for the growth of insect cells that is still in use. In the 38 years since that report, insect cell culture has expanded rapidly and changed from an area of research to a technology applicable in many fields. Because Grace used a medium supplemented with hemolymph, insect cell culture was limited at first to researchers who had access to insects. Development of media supplemented with commercially available materials, such as fetal bovine serum, made insect cell cultures widely available and permitted large volume culture. Today a number of media are available that require no supplementation. Further research resulted in cell lines from insects in many Orders and from other invertebrates. Perhaps the lines that have most impacted the field are the Tn-368 line from *Heliocoverpa zea* and the Sf-21 line from *Spodoptera frugiperda* which are easily cultured and replicate several baculoviruses. The genetic engineering of baculoviruses attracted commercial interest and financial support from the medical community, which resulted in rapid advances in media formulation and culture methodology. As this Tenth International Colloquium convenes, many of the tools necessary-the cells, the media, the equipment-for the use of insect cells in the areas of basic research, agriculture, and medicine are in place and the third Millennium looks promising.

I-2

Development of highly nutritive culture media. J. MITSUHASHI. Department of Bioscience,Tokyo University of Agriculture, Sakuragaoka 1-1-1, Setagaya-ku, Tokyo 156-8502, Japan. Email: junmths@nodai.ac.jp

One of the reasons why fail cells from certain invertebrates fail to grow is the inadequacy of culture media. Finding culture media suitable for the cells to be cultured is, therefore, important. MGM-450 medium has been used successfully to support growth of various cells or organisms. FRI-SpIm-1229, FRI-MntH-520A, and TUAT-SpLi-221 cell lines have been established with this medium. The MGM-450 medium also supported the growth of endoparasitoids, *Venturia canescens*, *Litomastix maculata*, and *Exorista sorbillans*, the best among tested media. MGM-464 medium was formulated by the further improvement of the MGM-450 medium, to develop a culture medium which can be used for initiation of primary culture of various invertebrates. With this medium, so far, the cells from *Pieris rapae crucivora*, and *Megacrania tsuda adan* were immortalized, several cultures from early embryos of *Bombyx mori*, and embryos of *Gastrophysa atrocyanea* became subcultured, and good cell migration was obtained in the primary cultures of the cells from ovaries of *Theretra japonica*, ovaries of *Cephonodes hylas*, and embryo of *Apis melliferra*. The composition of the medium and some growth of cells in primary cultures will be shown.

I-3

Novel techniques to establish new insect cell lines. DWIGHT E. LYNN, Insect Biocontrol Lab, USDA/ARS, Beltsville, MD 20705-2350. E-mail: dlynn@asrr.arsusda.gov

While 500 insect cell lines have been established over the past 35 years, most were developed using undifferentiated, generalized tissues (such as embryos, young larvae, or ovaries) and thus most of the cell lines are of an undetermined cell type. In the early 1980's, we successfully developed cell lines from lepidopteran wing imaginal discs. While these tissues are also undifferentiated, they are developmentally 'determined' to become wings in the adult. As such, they are mostly composed of epithelial cells and two of the three lines we successfully developed appear to be epithelial cells. In spite of this success, the only 'novel' aspect of this work was in selection of the tissue and meticulously dissecting it from the larvae, since we resorted to standard media, culture conditions and patience. In later work, I found that selection of cell colonies from primary or early passage cultures could separate cells with unique morphologies. More recently, I've been attempting to culture cells from the honey bee. This is the most widely domesticated insect, yet no cell cultures exists. One novel approach we have taken with this insect was based on the knowledge that, unlike most insects which use cholesterol as the precursor for steroid hormones, the honey bee uses a plant sterol, 24-methylene cholesterol. Unfortunately, while addition of this compound to cultures improved their longevity, it did not lead to establishment of a continuous cell line. Now we are using cell lines from other Hymenoptera as feeder cells to see if they will provide stimulatory factors to aid growth of the primary culture cells.

I-4

Pantropic Retroviral Vectors for Gene Transfer into Invertebrate Cells. J.C. BURNS[1], C. Shimizu[1], V. Boulo[2], and H. Shike[1]. [1]Dept. of Pediatrics, UCSD School of Medicine, La Jolla, CA 92093-0830; [2] DRIM, IFREMER-CNRS, Univ. Montpellier II, 34095 Montpellier, France. Email: jcburns@ucsd.edu

Pantropic vectors contain the envelope glycoprotein of the vesicular stomatitis virus (VSV-G), which binds to phospholipid moieties in the cell membrane to mediate vector attachment to the cell. Following attachment, the vector particle is endocytosed and the nucleocapsid is released into the cytoplasm following fusion of the VSV-G with the endocytic vesicle membrane at low pH. Reverse transcription mediated by the retroviral polymerase allows conversion of the viral RNA genome into double-stranded DNA. Pre-integration complexes form that contain the double-stranded DNA, viral protease, and viral integrase. These complexes are translocated to the nucleus during cell division when the nuclear membrane breaks down. Viral enzymes mediate integration of viral DNA into the host cell genome in regions of active transcription. Pantropic vectors have an extremely broad host cell range and can be concentrated to high titers ($>10^9$ cfu/ml) suitable for microinjection. To date, successful transfer and expression of foreign genes has been achieved in somatic cells of members of the phylum Mollusca (*Crassostrea virginica, Crassostrea gigas*) and the phylum Arthropoda (*Penaeus stylirostris*). Successful infection of embryos has been achieved in shrimp (*Penaeus stylirostris*) and mosquito (*Aedes triseriatus, Anopheles gambiae*). Transgenic organisms with documented foreign gene expression in the F_1 generation have been created in surfclam (*Mulinia lateralis*) and amoeba (*Entamoeba hystolitica*). The MoMLV U3 region of the 5' LTR is active as a promoter in many invertebrates including arthropods and molluscs. Internal promoters can also be used to express transgenes. Thus, pantropic retroviral vectors can transduce and mediate foreign gene expression in a wide variety of invertebrate species.

I-5

Slow and Fast Regulation of Corpora Allata in a Heteropteran Insect. M. HODKOVA[1], T. Okuda[2] and R.M. Wagner[3]. [1]Institute of Entomology, Czech Academy of Sciences, Ceske Budejovice, CZECH REPUBLIC; [2]National Institute of Sericultural and Entomological Sciences, Japanese Ministry of Agriculture, Fisheries and Forestry, Tsukuba, Ibaraki, JAPAN; and [3]Biological Control of Insects Research Laboratory, USDA, ARS, Columbia, MO. E-mail: magda@entu.cas.cz

Activity of corpora allata (CA) of the fire bug, *Pyrrhocoris apterus* (Heteroptera), was determined *in vitro* by measurement of the radioactive nonpolar compounds released into incubation medium containing ^{3}Hmethionine. The CA of adult females had little or no activity. Supplementation of farnesol specifically enhanced radioactivity in an unknown product (Un) that is different from the known juvenile hormones (JHs). The rate of production of Un by the CA in vitro was correlated with the effect of the CA on ovarian maturation *in vivo*. Therefore the Un could be a good candidate for the heteropteran JH that has not yet been identified. In vitro production of Un is regulated by the pars intercerebralis of the brain. Based on surgical interventions to the neuroendocrine system in vivo and *in vitro*, slow (irreversible *in vitro*) and fast (reversible *in vitro*) regulation of the CA can be distinguished. The slow stimulation or inhibition is associated with regulation of the CA volume. The fast inhibition is produced by the intact brain, while the fast stimulation is only produced by the brain extract.

I-6

Metamorphosis During the Passage of Drosophila Imaginal Disc Cell Lines — Cell Adhesion, the Effect of Insect Hormones and Ageing. M.J. MILNER, D.M. Cottam, and A.S. Miller. School of Biology, University of St. Andrews, St. Andrews, Fife KY16 9TS, UK. Email: mjm5@st-andrews.ac.uk

We have established culture conditions which enable partially dissociated fly imaginal discs to form primary cultures which may give rise to permanent cell lines. Cell lines undergo a form of morphogenesis during the course of a passage, first growing to confluence and then aggregating. This aggregation response is enhanced by the addition of the molting hormone 20-hydroxy ecdysone, which also causes cell elongation and cell death. Juvenile Hormone has been found to ameliorate the effects of 20-hydroxy ecdysone on cell growth. Cell lines at high passage numbers multiply faster, adhere less firmly to the substrate and loose the tendency to aggregate compared to young cell lines. We have examined cell-cell adhesion in roller cultures, and we note varying rates of adhesion at different parts of a passage, correlating with the stage of morphogenesis. We have produced cloned cell lines with selected characteristics such as reduced cell adhesiveness and a lack of response to molting hormone.

I-7

Juvenile hormone promotes the maintenance of lamellipodia in a lepidopteran cell line, and mimics the effects of signalling by lysophosphatidic acid and exogenous phospholipase D. S. DYBY, C. E. Leach, and H. Oberlander, Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, United States Department of Agriculture, Gainesville, FL 32604. Email: dyby@nersp.nerdc.ufl.edu

Juvenile hormone (JH), fenoxycarb, lysophosphatidic acid (LPA), exogenous phospholipase D, bombesin (a neuropeptide), and biogenic unsaturated fatty acids (linoleic acid, but not oleic acid) induce specific cell shape changes in the PID2 and the PID2A lepidopteran cell line. The percentage of cells that maintain broad lamellae (lamellipodia), i.e. flattened extensions, doubles with the application of juvenile hormone I, III, LPA, or PLD, from one to several days after test compounds are removed. The cells are sensitive to nanomolar concentrations of JHI. Complete Grace's medium is necessary to enhance the response to JH. This complete medium contains various growth factors, including transforming growth factor (TGF-beta) and LPA. Gundersen et al. (J. Cell Science 107: 645–659, 1994) and Cook et al. (J. Cell Biol. 141(10): 175–185, 1998) found that LPA, by activating the GTPase, Rho, as well as TGF-beta stabilize microtubules in NIH-3T3 mammalian cells. We suggest a model whereby juvenile hormone activates the signalling pathway controlled by extracellular LPA and preferentially promotes the uptake of the TGF-beta superfamily, such as dpp, into the cells.

I-8

Analysis of transcriptional regulation by ecdysteroids in ecdysone sensitive cultured cell line derived from the silkworm, *Bombyx mori*. S. TOMITA, A. Seino and S. Imanishi. Laboratory of Cell Engineering, Department of Insect Genetics and Breeding, National Institute of Sericultural and Entomological Science, Tsukuba, 305–8634, Japan. E-mail: tomita@nises.affrc.go.jp

Ecdysteroids coordinate insect molting and metamorphosis. The multivalent action of ecdysteroid is mediated by the ecdysone receptor complex, which triggers a cascade of transcription factors that direct molting and metamorphosis when activated by its ligands. The functional ecdysone receptor is the heterodimer of EcR and USP protein, which belong to nuclear hormone receptor superfamily. To analyze the molecular mechanism of ecdysone signal transducion in detail, we constructed reporter assay system using ecdysone sensitive cell line from the silkworm. Relative biological activities of several ecdysteroids are assessed. We expressed *Bombyx* EcR (BmEcR-B1) and *Bombyx* USP (BmUSP/BmCF1) as recombinant protein. Using purified proteins and [^{3}H]-Ponasterone A, we performed the kinetic analysis of the interaction between the receptor and ligand. Next, we revealed relative affinities of several ecdysteroids to the receptor by competitive assay and compared to the biological activities revealed by the reporter assays. We also performed kinetic analysis of the interaction between the receptor and DNA of its recognition sequence (ecdysone response element, EcRE) by using electrophoresis mobility shift assay and surface plasmon resonance sensor. Obtained kinetic parameters showed that 1) Interaction between the receptor and the ligand is rapid enough to mediate acute response to ecdysone. 2) Binding of the ligand does not affect the kinetics of the interaction between the receptor and EcREs.

I-10

Capabilities of Stem Cells from the Lepidopteran Midgut. R.S. HAKIM[1], M. Loeb[2], and K. Baldwin[1] 1. Dept. of Anatomy, Howard University 520 W St. NW, Washington D.C. 20059, 2. Insect Biocontrol Laboratory USDA, Beltsville MD 20705. E-mail: rhakim@fac.howard.edu

The growth of the larval lepidopteran midgut results from the proliferation and subsequent differentiation of a stem cell population that resides among the bases of the mature columnar and goblet cell types. In this location these cells are exposed to growth and developmental factors. While the timing of application of these factors is currently unknown,the major accumulation of stem cells occurs rapidly just prior to the molt. The subsequent differentiation of these cells within the molt period results in growth of the midgut. In vitro,stem cells collected immediately prior to the molt respond to two differentiation factors that have been identified and synthesized. In vitro, a role for stem cells in opposing the effects of BT damage to midgut cells has recently been demonstrated. It is not currently clear whether the capabilities of the stem cells taken early in an instar are identical to those taken immediately prior to the molt. These stem cells excised early in an instar and grown in vitro do, however, proliferate and differentiate in standard growth and differentiation media as do stem cells taken at the end of an instar. The proliferation characteristics of the stem cells taken from staged insects within an instar are currently being compared. The analysis of stem cell activity in vivo and in vitro is providing an overview of the capabilities in the midgut.

I-11

Cockroach Midgut Peptides and monoamines that regulate cell proliferation, Differentiation and Death. M. TAKEDA[1], T.Sakai[1], T. Tanigawa[1], Y. Yoshida[1], K. Iwabuchi[2] and M.J.Loeb[3]. 1Kobe Univ., 2 Tokyo A&M Univ., JAPAN.3 INSECT BIOCONTROL LAB, USDA, BELTSVILLE MD. E-mail:matakeda@kobe-u.ac.jp

Insect midgut cell numbers are maintained homeostatically. However, insects are often deprived of water and foodstuffs, where the rate of cell replacement must be severely affected. Insects also undergo metamophosis in which the internal organs are drastically remodeled by cell proliferation, differentiation and apoptotic processes. Ca.1500 midguts of Periplaneta americana were fractionated to obtain peptides that regulate these processes. The fractions were tested in midgut-derived tissue cultures from larval Heliothis virescens. Stem cells differentiate in vitro to either to columnar or goblet cells. Some fractions stimulated cell proliferation and differentiation while others caused loss of differentiated cells. One fraction induced a sharp reduction in cell number. The same series of fractions were also tested in last-instar larval fat body cell cultures of Mamestra brassicae. Some fractions stimulated cell proliferation. In the case of constitutive replacement of epithelial cells, we investigated the 5HT content with respect to starvation and refeeding, since 5HT-like immunohistochemical reactivity was observed in the nidi, the nests of regenerative (stem) cells. 5HT decreased when roaches were starved. Transplantation of hemolymph from the fed roaches and injection of 5HT into the starved animals both stimulated BrdU uptake in the nuclei of the cells in the nidi. 5HT somehow stimulated cell t. This is the first report showing involvement of the IP3 signaling cascade in a member of the Na+/Cl- dependent neurotransmitter transporter. The ability of a transporter protein to activate intracellular signaling suggests these transporter proteins may have more diverse functions than previously believed.

I-12

Control of Life and Death, with Indications for Midgut Repair in Cultured Midgut Cells from the Lepidopteran, *Heliothis virescens*. M. J. LOEB, Insect Biocontrol Laboratory, US Dept. of Agriculture, Beltsville, MD 20705. Email: mloeb@asrr.arsusda.gov

Primary Cultures of *Manduca sexta* and *Heliothis virescens* midgut arise from stem cells that migrate from tissue fragments to generate a culture of morphologically distinguishable stem, columnar, goblet, and occasional nerve cells. The stem cells divide and differentiate to replenish the supply of mature cells that die by apoptosis. Isolated stem cells require fat body extract to mitose, but do not differentiate. Two factors isolated from conditioned medium, MDF1 and MDF2, induce stem cell differentiation to mature columnar and goblet cells. In adverse conditions, the rate of apoptosis can increase from 7 to 50%; the proportion of apoptosing stem and differentiating cells increases, indicating down regulation of the cells that replace lost mature cells. Under severe conditions, such as a dose of Bt toxin, many mature cells die but the number of stem and differentiating cells increases dramatically. When the toxin is washed away, the culture repopulates to its original composition. This activity parallels a non-fatal Bt toxin exposure or virus infection *in vivo*, where the midgut is repaired. Since only midgut cells are present in culture, the factors that regulate the rate of apoptosis, mitosis, differentiation, and organization must be synthesized by those midgut cells.

I-13

Transport processes in the insect midgut. S. S. GILL. Dept. Cell Biol. & Neuroscience, Univ. California, Riverside, CA 92521. E-mail: Sarjeet.gill@ucr.edu

The insect midgut, particularly in lepidopteran insects, is a highly energized tissue. This energization, which occurs primarily from transport of protons by the V-ATPase in the goblet cell apical membrane, drives the movement of nutrients into columnar cells. The uptake of many nutrients including amino acids also co-transports potassium ions. Consequently the hemolymph of lepidopteran insects has high levels of potassium. The potassium is transported back into the gut lumen through the goblet cell facilitated in part by a newly identified transporter-like protein inebriated. Phylogenetic analysis shows this protein is divergent from other neurotransmitter transporters, suggesting it probably has a distinct function from that of the other transporters. Using heterologous expression in Xenopus we show that oocytes expressing inebriated do not transport any of the commonly known ligands but respond to hyperosmotic stimulation, which causes the release of intracellular Ca2+. In Xenopus oocytes this release stimulates endogenous calcium-activated Cl- currents. This Ca2+ release requires the N-terminus and occurs via the IP3 signaling pathway. Immunohistochemical analyses show Manduca inebriated is distributed in tissues involved in potassium transport, including the goblet cells in the midgut, the Malpighian tubules, the hindgut and the central nervous system. We propose that stimulation of inebriated releases intracellular Ca2+ in native tissues, activating Ca2+-dependent K+ channels, leading to K+ transport. This is the first report showing involvement of the IP3 signaling cascade in a member of the Na+/Cl- dependent neurotransmitter transporter. The ability of a transporter protein to activate intracellular signaling suggests these transporter proteins may have more diverse functions than previously believed.

I-14

Primary and established midgut cultures from *Pseudaletia unipuncta* and *Trichoplusia ni* larvae for baculovirus studies. R.R. GRANADOS, J. Zhong, G. Li, J. Garcia and P. Wang. Boyce Thompson Institute, Cornell University, Ithaca, NY 14853-1801. E-Mail: rg28@cornell.edu

Midgut epithelial cells were isolated from mid-fourth and fifth instar *Trichoplusia ni* and *Pseudaletia unipuncta* larvae, respectively, by collagenase treatment of midgut tissues, and cultured in TNMFH medium. The primary cultured cells remained viable for over 4 days and were suitable for binding and fusion studies with the *Autograph californica* nucleopolyhedrovirus. Long term continuous culture and establishment of a midgut cell line was achieved with the *P. unipuncta* armyworm intestinal cells. Several cell lines were obtained from these armyworm primary cultures and have been subcultured and maintained for over 18 months. The three major cell types are present in cultures and include stem cells (regenerative cells), columnar, and goblet cells. Morphogenesis of columnar and goblet cells from stem cells was followed *in vitro*. There appears to be a cycle of apoptosis of goblet and columnar cells and their replacement by stem cells. After approximately six passages, the cell density in T-flasks appeared to be somewhat constant, reaching 10^3 and 10^4 cells per ml of medium. Insulin, heparin, and 20-hydroxyecdysone did not stimulate cell proliferation. The columnar cells are round to rectangular in shape and possess a brush border while the goblet cells have a classic flask-like shape with a central cavity. The midgut cells in culture grow in suspension but some success was achieved in the attachment of the cells to culture wells coated with the cell attachment factors, collagen or fibronectin. Infection of these cells with baculoviruses will be described.

I-15

In Vitro Hemocyte and Tissue Assays for Assessing Baculovirus Interactions with their Host Insects. J.O. WASHBURN, D. Trudeau, E. Haas-Stapleton, and L.E. Volkman. Dept. of Plant and Microbial Biology, University of California, Berkeley, CA 94720-3102. E-mail: janwash@nature.berkeley.edu

Autographa californica M nucleopolyhedrovirus (AcMNPV) is the type species of the Baculoviridae, a family of arthropod specific viruses that infects primarily insects. AcMNPV is known to kill the larvae of more than 30 species of Lepidoptera, but among hosts resistance to fatal infection is highly variable. Utilizing recombinants of AcMNPV outfitted with reporter genes, we have found a common pathway whereby infections are initiated and spread within caterpillar hosts. Primary infection occurs within columnar midgut cells, and secondary infections are initiated first within the insect's respiratory (tracheal) system; subsequently, infections are spread throughout the insect by tracheal epidermal cells and by hemocytes circulating within the hemolymph. In some resistant hosts (e.g., *Helicoverpa zea*, *Manduca sexta*), however, shortly after budded virus leaves the midgut and enters the tracheal epidermis, hemocytes aggregate at infection foci and the spread of virus is halted. In other host species, viral gene expression is rarely observed within the midgut, and secondary infections are not established. In order to help elucidate how viral infections are blocked within specific tissues, we have developed protocols for separating the larval midgut epithelium from its basal lamina and associated tracheal elements and for culturing the isolated tissues in vitro. These techniques, coupled with in vitro studies using hemocyte cultures, allow us to evaluate the permissiveness of specific host tissues, thereby providing new insights into the mechanisms by which larval lepidopterans block AcMNPV infections.

P-1

Cryopreserved Storage of Clonal Germplasm: the promise is fulfilled. B.M. REED. USDA-ARS National Clonal Germplasm Repository, Corvallis, OR 97333-2521 USA reedbm@bcc.orst.edu

Plant tissue cryopreservation began in the 1970's, developed in the 1980's, and finally came of age at the end of the century. The first meristem cryopreservation experiments of the 1970's were followed by the development of several highly successful protocols in the 1980's. By the early 1990's some plant germplasm was stored as apical meristems of in-vitro cultured plantlets, and as dormant buds from dormant temperate tree-fruit crops. Storage protocols, record management, and related issues are now in place at several genebanks and genetic resources laboratories. Cryopreserved base collections are now available for apple (China, US), coffee, palm (France), hops (Spain, US), pear, blackberry, grass, hazelnut (US), potato (Germany), currants and gooseberries (UK, US) and may soon be in place for cassava (Columbia), strawberry, grape (US), and sugarcane (Cuba). These base-storage collections of clonal germplasm provide security for safeguarding long-term access to genetic diversity that is vital to the continued improvement of many clonally propagated crops.

P-2

ADVANCES IN CRYO-STORAGE FOR ROOT AND TUBER CROPS Ali M. Golmirzaie1 and Ana Panta2. 1College of Agriculture. Department of Horticulture, 316 Pant Sciences Building, University of Arkansas, Fayetteville, Arkansas 72701. E-mail: amgolmir@comp.uark.edu 2International Potato Center, P.O.Box 1558-Lima 12, Perú. E-mail: a.panta@cgiar.org

Ex-situ conservation of several rot and tuber crops is the main responsibility of the International Potato Center (CIP). CIP maintains the largest worldwide potato and sweetpotato collections under field and in vitro conditions. To assure a safeguarding of this material, a continuous improvement of conservation methods is necessary. In 1995, CIP started testing the cryopreservation techniques by vitrification, a method developed by P. Steponkus. The goal of this project was to reduce the cost and labor involved in the conservation of the collection. Up to date, the potato cryo-collection maintained at CIP comprises 390 accessions (including 9 species). Viability evaluations of 103 accessions stored in liquid nitrogen for 1 to 2.5 years was 38%. Assays to test the genetic stability are underway. To increase the survival rate, CIP is working to improve the vitrification process, for example, testing alternative dehydration solutions. The experience obtained on potato has paved the way to start the research on sweetpotato cryopreservation. After testing several methods, a protocol based on Pennycooke and Towill's vitrification method was defined as the most promising. Nine genotypes representing a wide diversity are being tested with this protocol. So far, survival rate observed with 4 genotypes was 42%. Pennycooke, J.C. y L.E. Towill. 1997. Cryopreservation of sweetpotato shoot tips by vitrification. HortScience, 32(3): 471–472. Steponkus, P.L., R. Langis and S. Fujikawa. 1992. Cryopreservation of plant tissues by vitrification. Advances in Low-Temperature Biology 1:1–16.

P-3

Cryopreservation as an Integral Part of Forest Genetics Programs. D.R. CYR. Silvagen Inc., Vancouver, BC, Canada V6S 2L2. E-mail: dcyr@Silvagen.com

In vitro propagation technologies (e.g., somatic embryogenesis, organogenesis) are important tools for the selection and deployment of rare or elite genotypes in forestry. Cryopreservation allows the production of plants following long-term trials required for tree species; this is essential for the successful implementation of propagation strategies in forest genetics programs. However, the efficacy of cryopreservation varies with the propagagation approach, which in turn depends on the target species. Currently, conifer somatic embryogenesis possesses the most advanced cryopreservation program, with large-scale efforts underway internationally. For example, at Silvagen Inc. more than 8,000 embryogenic lines representing 15 conifer species have been stored via cryopreservation since 1992. The management issues associated with this germplasm bank include integration with clonal selection programs and the routine provision of fresh cultures to research programs. Focus in this presentation will be placed on the mechanisms and challenges associated with implementation and risk-management as it applies to these roles.

P-4

Recent advances in cryopreservation of fruit germplasm. DAMIANO C.°, F. Engelmann°° and A. Frattarelli°. ° Fruit Tree Research Institute– 00040 Ciampino Aeroporto. Italy. E-mail: isf.propag@mclink.it. °° International Plant Genetic Resources Institute, via delle Sette Chiese, 142-00145 Roma. Italy E-mail: f.engelmann@cgiar.org

Genetic resources of fruit crops are traditionally maintained ex-situ through field collections, which are located in areas whose climatic conditions are those needed by the species. However, field collections remain exposed to natural disasters, attacks by pests and pathogens, which can wipe them out, and their maintenance costs are very high. Cryopreservation is the only technique which ensures the safe, cost-effective and long-term conservation of genetic resources. Research on fruit crop cryopreservation has been initiated some years ago and nowadays the interest for its application is increasing world-wide. The routine application of cryopreservation in genebanks is still restricted to a limited number of species, although a number of the major fruit-species are being studied. Among them strawberry, almond, apple, kiwi, peach and walnut are under investigation in our Institute. So far, strawberry cells have been successfully stored using pretreatment with a vitrification solution followed by two-step freezing. For all the other species, encapsulation and vitrification of apices have been compared; higher survival has been consistently obtained using encapsulation, which has been adapted to the material case by case. To speed up the routine application of cryopreservation it seems essential to give more attention to fundamental aspects including the physiology of cell and tissue dehydration, genetic stability and to the related molecular markers. Finally, there is an increasing number of cases where cryopreservation can be implemented after minor adaptations of the protocols.

P-5

Newly Developed Encapsulation-Dehydration Protocol for Plant Cryopreservation of *In Vitro*-Grown Apices. A. SAKAI [1*], T. Matsumoto[2], D. Hirai[3], R. Charoensub[4], and T. Niino[5]. 1—Hokkaido University (retired), (Home Address) Asabucho 1-5-23, Kitaku, Sapporo, 001-0045 JAPAN; 2—Shimane Agricultural Experiment Station, Izumo, Shimane, 693-0035 JAPAN; 3—Hokkaido Prefectural Plant Genetic Resources Center, Takikawa, 073-0013 JAPAN; 4—Scientific Equipment Center, Kasetsart University Research and Development Institute, Bangkok, 10900 THAILAND; 5—Department of Uplands Farming, Tohoku National Agricultural Experiment Station, Fukushima, 960-2156 JAPAN. Email: asakai@mxumeshnet.or.jp

Vitrification and the encapsulation-dehydration technique are potentially valuable cryogenic protocols for apices. In the vitrification protocol, explants are sufficiently dehydrated osmotically at 25 or 0°C by a highly concentrated vitrification solution prior to a plunge into LN. Our vitrification protocol has been successfully applied to more than 140 plant species or cultivars in both temperate and tropical origins. These results strongly suggest that the vitrification is a sophisticated and very valuable protocol for cryopreserving apices. However, the protocol needs to use a highly concentrated vitrification solution. Thus, the duration of dehydration with the vitrification solution has to be very precisely timed. It is also impossible to manipulate many samples at the same time. In the encapsulated dehydration technique, encapsulated apices are osmoprotected with 0.8 M sucrose for one day before air-drying. This technique allows much more flexibility for handling large amounts of materials than vitrification. However, the problems are a lower rate of recovery growth and a longer dehydration process when compared to the vitrification protocols. We presented a simplified and efficient protocol, which potentially combines all advantages of two cryogenic techniques of vitrification and encapsulation-dehydration. In this protocol, during the encapsulation process, the apices precultured with 0.3 M sucrose for 16 h were effectively osmoprotected with a mixture of 2 M glycerol plus 0.4 M sucrose as in our vitrification protocol. They were directly dehydrated with dry silica gel prior to a plunge into LN. This protocol produced much higher rates of recovery growth in the four plant species (including cassava) tested than those cryopreserved by the conventional encapsulation-dehydration. This protocol also considerably reduced the time needed for the cryogenic procedure. In addition, this technique allows much more flexibility for handling large amounts of materials when compared to vitrification.

P-6

Development of Transgenic Plants for Ecotoxic Mercury Degradation and Removal. C.L. RUGH, S.A. Merkle and R.B. Meagher. Michigan State University, East Lansing, MI 48824 and University of Georgia, Athens, GA 30602. E-mail: rugh@msu.edu.

We have employed a biotechnological approach to genetically engineer plants with bacterial genes encoding enzymes for detoxification of reactive and harmful mercurial compounds. A variety of plant species, including laboratory model plants such as *Arabidopsis* and tobacco, were regenerated with modified forms of the bacterial genes, *merA* and *merB*. Transgenic Mer-plants survived upon levels of mercurial compounds that were highly toxic to untransformed plants. Mercury resistance in the transgenic plants was confirmed to be via enzymatic conversion of ionic and organic mercurials to far less reactive and greatly detoxified elemental mercury, which rapidly evaporates from their tissues to naturally dispersed levels in the atmosphere. These capabilities were observed in aqueous, agar gel, and soil media, offering this technology as a potentially less expensive and more environmentally compatible alternative to current methods of remediation of mercury contaminated habitats. Recent production of *mer*-transformed wetland plant species allows treatment of the most widespread and hazardous areas of mercury pollution. Anaerobic wetland and riparian sediments are the sites for production of methylmercury, one of the most ecotoxic compounds known, largely due to its tendency to bioaccumulate at higher trophic levels. Mer-plants can directly degrade these substances *in situ*, and simultaneously use excess water and aerate soils to block further microbial synthesis of methylmercury. This research demonstrates the feasibility for utilization of foreign genes to confer novel abilities to plants for beneficial environmental applications.

P-8

Physiology, Biochemistry and Molecular Biology of Metal Hyperaccumulation in Plants. D.E. SALT, Chemistry Department, Northern Arizona University, Flagstaff, AZ 86011. Email: david.salt@nau.edu

Certain plants, growing in their native habitat, have the extraordinary ability to accumulate high concentrations of particular metals in their shoots, including Ni, Zn and Se. The genetic material underlying this trait is clearly valuable for the development of plants suitable for phytoremediation. The ability to control the uptake, accumulation and biotransformation of these metals in plants would also have a major impact on improving the nutritional properties of certain food crops. For these reason we have been investigating the physiological and biochemical mechanisms which underlie this hyperaccumulation phenotype. A comparison of root exudates, Ni transport rates and Ni tolerance in the Ni hyperaccumulator *Thlaspi goesingense* and the non-accumulator *Thlaspi arvense* has revealed that the primary mechanism underlying the Ni hyperaccumulation phenotype in *T. goesingense* is an enhanced cellular Ni tolerance mechanism. In order to understand the mechanisms involved in this enhanced Ni tolerance we have investigated both the in planta speciation and the cellular localization of Ni. Using X-ray absorption spectroscopy (XAS) we have determined what ligands are involved in binding Ni within these two Thlaspi species. In the hyperaccumulator the majority of intracellular shoot Ni appears to remain complexed with citrate where as in the non-accumulator histidine-like ligands appear to play an increasing role as Ni accumulates to toxic concentrations. We have also demonstrated that *T. goesingense* has an enhanced ability to accumulate Ni in the vacuoles of shoot cells, when compared to *T. arvense*. Both the chemical speciation and localization data therefore support the conclusion that Ni tolerance in shoots of *T. goesingense* is mediated by the efficient accumulation of Ni-citrate within the vacuole. However, the question remains as to what is the molecular mechanism of this enhanced vacuolar accumulation. To address this we are investigating the role of both putative shuttle-molecules involved in carrying Ni across the cytoplasm to the vacuolar membrane, and tonoplast transport proteins involved in pumping Ni into the vacuole.

P-9

Defusing the environment; engineering plants to degrade explosives. N.C. BRUCE. Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QT. United Kingdom. E-mail: n.bruce@biotech.cam.ac.uk.

Widespread contamination of the environment by explosives resulting from the manufacture, disposal and testing of munitions is becoming a matter of increasing concern. Most explosives are considered to be a major hazard to biological systems due to their toxic and mutagenic effects. Interest on the bioremediation of land contaminated with explosives has recently been focused on phytoremediation. The capital cost of using plants is low (several hundred times less expensive than the equivalent amount of bacterial biomass), ongoing operational costs are minimal, implementation is easy and non-invasive, healthy plants are capable of producing up to 300 million miles of root per hectare, and public acceptance is high. Unfortunately, whilst plants have many advantages for the remediation of contaminated land and water they lack the catabolic versatility which enables microorganisms to mineralise such a wide diversity of xenobiotic compounds. This raised the interesting question as to whether the impressive biodegradative capabilities of soil bacteria could be combined with the high biomass and stability of plants to yield an optimal system for *in situ* bioremediation of explosive residues in soil. Our investigation into the degradation of explosive residues by soil bacteria resulted in the isolation of *Enterobacter cloacae* PB2, which is capable of utilising nitrate ester explosives, such as pentaerythritol tetranitrate (PETN) and nitroglycerin, as the sole source of nitrogen for growth. Denitration is effected by an NADPH-dependent enzyme designated PETN reductase. Importantly, PETN reductase was shown to be capable of degrading the aromatic explosive 2,4,6-trinitrotoluene (TNT), probably the most persistent pollutant in military sites, via a reductive pathway resulting in nitrogen liberation. We have successfully introduced PETN reductase into plants to create transgenic plants that degrade explosives. Since the bacterial degradative pathways for many classes of organic pollutant have been elucidated, this may be a generally applicable method of achieving bioremediation of contaminated soil in the environment.

P-10

Micropropagation, in vitro conservation and characterization of chayote (Sechium edule). Abdelnour, A1., Rocha, O2. and Engelmann, F3. 1. Institute of Technology, Cartago, Costa Rica. Mail to: raguero@cariari.ucr.ac.cr 2. School of Biology, University of Costa Rica Mailto: ojrocha@cariari.ucr.ac.cr 3. IPGRI, Rome, Italy Mailto: f.engelmann@cgiar.org

Chayote (Sechium edule), (Cucurbitaceae), is an important staple food in Mesoamerica, the Caribbean and Tropical South America. It is one of the less expensive vegetables available, and every part of the plant is useful. Among the main factors limiting production are availability of high quality planting material and sanitary problems, especially viruses. Intense selection of phenotypes suitable for international markets has rapidly increased genetic erosion during the last 30 years. The present study attempts to develop protocols for production of virus-free vegetative planting material and for long term conservation of germplasm, using tissue culture and cryopreservation. In addition, a field collection of about 60 accessions recently established in Costa Rica is being characterized using isosymes and RAPDs techniques. Plant regeneration from meristems and micropropagation protocols have been established using shoots as starting material, but meristem culture did not result in virus-free plants. Combinations of meristem culture with thermal and chemotherapy are being evaluated. Cryopreservation of zygotic embryos using dehydration-rapid freezing in liquid nitrogen has been successfully conducted and encapsulation-dehydration techniques are being tested with shoots. For genetic characterization, we have established the protocol for 8 enzyme systems (IDH, MDH, PGI, PGM, PGD, SKD, SOD, PER). Two buffer systems, histidine/citrate and citrate/histidine, provide excellent resolution for ten putative loci.

P-11

In Vitro Shoot Regeneration of Several *Citrus* Species. M.N. NORMAH. School of BioSciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM, Bangi, Selangor, Malaysia.E-mail: normah@pkrisc.cc.ukm.my

In vitro propagation of tropical fruits such as *Citrus* serves two purposes; mass propagation and regeneration of cryopreserved material. Emphasis is given to wild/endangered species of *Citrus* and those with recalcitrant (low temperature and desiccation sensitive) seeds. Explants such as hypocotyls, nodes and shoot tips were obtained from *in vitro* germinated seedlings. Multiple shoot formation was obtained from hypocotyl, node and shoot tip explants of *Citrus halimii* cultured on Murashige and Skoog medium supplemented with 0.4–11.1μM 6-benzyladenine. Hypocotyl was the best explant for multiple shoot regeneration. Maximum number of shoots was obtained on medium with 2.2–11.1μM 6-benzyladenine. For shoot tip culture, single and multiple shoot regeneration was observed. The maximum number of shoots (3.7) was obtained on medium supplemented with 4.4–11.1μM 6-benzyladenine. The shoots were successfully rooted and transferred to the soil mixture consisting of soil, sand and organic material (1:1:1) and kept in the glasshouse. Similar results were obtained with *Citrus hystrix*, *Fortunella polyandra* and *Murraya koenigii*.

P-12

In Vitro Propagation of Tropical Forest Trees. ENNY SUDARMONOWATI. R&D Centre for Biotechnology-Indonesian Institute of Sciences, JI Raya Bogor KM 46, PO Box 422, Bogor, INDONESIA. Email: s_enny@hotmail.com

The availability of good quality planting materials of forest tree species is the main problem in the tropical countries which encounter high rate of deforestation. In order to solve this problem, rapid propagation techniques such as tissue culture or *in vitro* propagation which are able to provide a large number of uniform planting materials are prerequisite. Unlike other plant species, the progress of *in vitro* propagation of tropical forest trees has been relatively slow as it is hindered by hight content of phenolic compunds. The number of the trees that could be propagated *in vitro* is very limited. Most technique used is shoot multiplication as somatic embryogenesis is very much influenced by many factors. *Acacia mangium, Eucalyptus urophylla* and *Tectona grandis* are among the species that have been propagated. On a large scale basis and some of them are still being tested in the field. Embryogenic callus had been produced from certain species such as *Shorea pinanga* and *Pometia pinnata*. Recent development of *in vitro* technique for propagating tropical forest tree species, for preserving the plantlets produced and for improving the propagated species are discussed.

P-13

Studies on *Amorphophallus titanum* Tissue Culture. IRAWATI. Treub laboratory, Ir.H. Juanda 22, Bogor 16122, Indonesia. Email: irawati@indosat.net.id

Explant from lateral buds of field-collected *Amorphophallus titanum* corm were cultured on MS and Gamborg media containing 0.1 ppm Naphthalene Acetic Acid (NAA) and 1 ppm Benzyl Adenine (BA), with or without 1 ppm 2,4-D or 0.1 ppm NAA and 0.01 ppm Kinetin. Addition of activated charcoal on 3 different multiplication media did not improved shoot and root formation. When different types of explants were inoculated on MS medium containing different concentrations of trans-Cinnamic Acid (t-CA) and Kinetin, single or multiple plantlets grew from shoot tip, however root development were limited on cultures originated from calli, root or petiole segments. Cultures on MS medium containing 0.3 ppm NAA and 0.03 ppm Zeatin gave the best result for plantlet/s and corm development compared to other MS media with coconut water, NAA + 2-iP, NAA + Kinetin, NAA + BA, 2,4-Dichlorophenoxyacetic acid or trans-Cinnamic acid.

P-14

Tracheary element formation: a molecular analysis M.C.MCCANN, D. Milioni, N.J. Stacey, P. Sado, M. Mourelatou, C. Domingo and K. Roberts Department of Cell Biology, John Innes Centre,Norwich, NR4 7UH, UK maureen.mccann@bbsrc.ac.uk

Mesophyll cells from the leaves of Zinnia elegans cv. Envy trans-differentiate into tracheary elements (TEs) when cultured in a medium containing a 1:1 ratio of auxin to cytokinin. This model system is unique among in vitro systems because of the readily inducible synchronous trans-differentiation of up to 80% of the cell population, making it ideal to study the processes involved in de-differentiation, commitment to a new cell fate, and the signal transduction events leading to differentiation and programmed cell death. In order to get a broad picture of the range of genes involved in this complex process, we require sequence information for comparison with known genes in databases and must identify both high- and low-abundance transcripts. We have recently applied a novel method of RNA fingerprinting to allow the detection of DNA fragments derived from RNA using cDNA synthesis and subsequent PCR-amplified fragment length polymorphisms (cDNA-AFLP). We have used cDNA-AFLP to screen the patterns of expression of about 35000 genes at time-points of 0h, 30min, 4h, 24h and 48h after hormone addition into the culture. We selected over 500 fragments whose transcription showed overt changes in abundance over time and obtained partial sequences of these. Database searches allow us to ascribe a putative identity to about 55% of the predicted gene products, although some are to genes of unknown function. About 10% of the partial sequences show no identity to any sequence in the databases. We are currently characterising the hormonal regulation and tissue-specific expression of these fragments by in situ hybridisation.

P-15

Recent Update in Cellulose Biosynthesis and Structure. R. MALCOLM BROWN, JR. Section of Molecular Genetics and Microbiology, The University of Texas at Austin, Austin, Texas 78712. Email: rmbrown@mail.utexas.edu

Cellulose is the most abundant macromolecule on Earth with some 10^{11} tons produced and destroyed annually. It is the major component of vascular plants as well as some fungi, algae, several bacteria, and animals (tunicates). Cellulose is a homopolymer of ß-1,4 linked glucans. Multiple polymers aggregate and crystallize to form the microfibril, the basic structural unit. Microfibrils are the major components of the cell wall. As such, they have a tremendous influence on plant cell growth and development. This review will cover new aspects of cellulose structure including atomic and molecular imaging of glucan chains and their crystalline order, and the sub-allomorphs of cellulose. Recent biochemical investigations will be introduced including *in vitro* cellulose assembly, purification and identification of enzymes. Molecular biology of cellulose will be covered including identification of genes for cellulose biosynthesis in bacteria and vascular plants, 3-d structure of cellulose synthase. Strategies for introduction of cellulose synthase genes into important crop plants are important for expanding the usefulness of this product. A major source of cellulose is cotton. A novel submerged cotton ovule method which yields cotton fibers with unique properties will be presented. Comments on the usefulness of cell culture in tackling difficult questions of cellulose assembly will be made. The presentation will conclude with predictions for the future and novel sources of cellulose, including synthetic cellulose production.

P-16

Exciting World of Plant Cellulose Synthases. C. P. JOSHI, L. Wu, R. V. Chavli and V. L. Chiang. Plant Biotechnology Research Center, School of Forestry and Wood Products, Michigan Technological University, Houghton, MI 49931. Email: cpjoshi@mtu.edu.

Cellulose biosynthesis is a major contributor to earth's biomass. Recent molecular genetic studies have suggested that the enzyme cellulose synthase (CesA) plays a major role in this process. In order to gain better insights in the structure, expression patterns, regulation, function and possible evolution of CesA genes from herbaceous plants and trees, we have cloned many CesA cDNAs from Arabidopsis and aspen which are members of a large multigene family. A typical CesA protein is predicted to have eight transmembrane domains, a Zinc-binding N-terminal domain for possible protein-protein interaction and a central cytoplasmic UDP-glucose binding domain encompassing the catalytic sites. By *in situ* hybridization using gene-specific probes, some Arabidopsis CesA transcripts were found to be distributed in all living tissues whereas aspen CesA transcripts were localized exclusively to developing xylem tissues. Transgenic plants carrying specific CesA promoter-GUS fusions corroborated these tissue-specific expression patterns of CesA genes. Interestingly, the aspen CesA promoter was also found to be responsive to mechanical stress conditions in addition to being developing xylem-specific. Compilation and amino acid sequence analysis of over 40 publicly available CesA and CesA-like (CSL) proteins from plants have indicated that the catalytic domain of CesA proteins in plants has evolved by multiple insertions of gene-family-specific sequences in the coding regions and the primitive CesA members are more similar to prokaryotic CesA proteins without such insertions. Preliminary results with genetic manipulations of CesA genes in transgenic tobacco and aspen plants will be discussed.

P-19

Engineering of Plant Secondary Metabolism for Human Nutrition and Health. B. McGONIGLE. Dupont Nutrition & Health, Experimetnal Station, Wilmington, DE 19880-0402. E-mail: brian.mcgonigle@usa.dupont.com

Isoflavones have been of significant interest due to their potential for promoting human health. We have cloned the gene encoding the enzyme responsible for the initial step of isoflavone biosynthesis isoflavone synthase (IFS) using an EST expression strategy. Using RT-PCR we have cloned IFS from eight other legume species as well as the non-legume sugarbeet. Isoflavone accumulation in soybean seeds is variable depending on both the cultivars of soybean and the environmental conditions under which they are grown. We have used stable gene expression in somatic embryos as a model system for transgenic soybean seed development. A developmental profile of isoflavone accumulation was developed in the somatic embryos and used to determine the proper sampling time for isoflavone accumulation. We expressed IFS in the somatic embryo system and detected isoflavone levels approximately two times higher than in wild type embryos. We have gone on to introduce an IFS coding region regulated by a seed storage gene promoter in soybean plants. Another aspect of our work is expression of IFS in non-isoflavone producing plants. Expression of soybean IFS in Arabidopsis results in the accumulation of genistein in Arabidopsis leaves. Soybean IFS has also been expressed in tobacco plants and BMS cells.

P-20

Producing Novel Seed Oils in Transgenic Plants. J. METZ, K. Lardizabal and V. Knauf. Calgene Campus of Monsanto, 1920 Fifth Street, Davis, CA 95616, USA. E-mail: jim.metz@monsanto.com

In contrast to the diversity found in nature, commercially developed crops produce a very limited set of fatty acids in their seed oils. Efforts to produce exotic oils in large volumes have centered on domestication of wild species and, more recently, on the introduction of foreign DNA into established crop plants using genetic engineering. The last few years have seen rapid advancements in the genetic engineering approach. We will provide an update on several projects initiated at Calgene to produce novel oils in Brassica using cDNAs derived from sources such as the California Bay tree, coconut, mangosteen and a fungus (*Mortierella alpina*). In addition, we will describe our efforts to introduce a wax synthesis pathway from jojoba (*Simmondsia chinensis*) into high erucic acid rapeseed. The key enzymes required for synthesis of these waxes are: an alcohol-forming fatty acyl-CoA reductase (FAR), a fatty acyl-CoA: fatty alcohol acyl-transferase (wax synthase; WS) and an efficient fatty acyl-CoA elongation (FAE) system. The FAE component is required due to the preference of the FAR and the WS for very long chain acyl-CoA substrates. The cDNA encoding the jojoba FAR and WS, as well as a key component of the FAE system have all been cloned. We are now in a position to produce these waxes in transgenic plants. Although jojoba oil has chemical and physical properties which make it potentially useful in a wide range of industrial applications, its high cost has limited its use to primarily cosmetic applications. It will be interesting to see what applications can be found for jojoba-like waxes produced in Brassica at a much lower cost.

P-21

Plantibodies for Reproductive Health: Herpes Therapy and Prevention. K.K. BRIGGS, L. Zeitlin, F. Wang, L. Chen*, J. Fitchen, J. Glynn, V. Lee, S. Zhang*, K. Whaley. Epicyte Pharmaceutical Inc., San Diego, CA 92121. *Novartis Agricultural Discovery Institute Inc., La Jolla, CA 92037. E-mail: kbriggs@epicyte.com

Herpes is an incurable sexually transmitted disease (STD) caused by the herpes simplex virus (HSV). Most cases of genital herpes are caused by HSV-2. In the United States, 22% of people 12 years and older are infected with HSV-2, with an estimated 1 million new infections each year. In addition, HSV can cause potentially fatal infections in the immunocompromised and in infants infected during delivery. If used, latex condoms offer incomplete protection against infection. We are developing an over-the-counter topical product that contains an anti-HSV antibody for protection against vaginal transmission of HSV. In addition, we are producing an anti-HSV antibody that will be used to treat infected neonates. Plant-based monoclonal antibody production allows the generation of virtually unlimited quantities of antibodies at prices that are 25 to 100 times less expensive than mammalian cell culture, while reducing manufacturing capital by 90%. The human sequences encoding anti-HSV antibodies as an IgG, IgA, dIgA, and sIgA were introduced into rice cells. Transgenic cells were selected and regenerated into plants. The majority of rice lines assayed produced assembled antibody. The rice-produced antibodies were able to effectively neutralize HSV-2 in standard *in vitro* neutralization assays. Furthermore, the rice-produced antibodies were able to prevent vaginal HSV-2 infection in mice. For over-the-counter applications, plants may offer the only method for producing protective antibodies cost-effectively and in sufficient quantities to be employed worldwide.

P-22

Oral Delivery of Subunit Vaccines using Corn STEPHEN J. STREATFIELD[1], Joseph M. Jilka[1], Debra Turner[2], Jocelyne Mayor[1], Michele Bailey[1], Susan Woodard[1], Leigh Anne Massey[1], Michael Horn[1], Donna Delaney[1], Elizabeth E. Hood[1], John D. Clements[3], Ian R. Tizard[2] and John A. Howard[1]. [1]ProdiGene, 101 Gateway Boulevard Suite 100, College Station, TX 77845. E-mail: sstreatfield@prodigene.com [2]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX 77843. [3]Department of Microbiology and Immunology, Tulane University Medical Center, 1430 Tulane Avenue, New Orleans, LA 70112.

Advances in the development of subunit vaccines and in the production of foreign proteins in plants together offer the prospect of stable and cheap vaccine delivery systems. Several groups have produced various bacterial and viral proteins in plants and have shown that these proteins are stable and can elicit immune responses in feeding trials. We have extended this approach to using corn as the plant production system. Corn has several advantages as a vaccine delivery vehicle, most notably established technologies to generate transgenic plants, to optimize traits through breeding programs and, if necessary, to process the seed into a palatable form. Here we report on the production in corn seed of the GM1 receptor binding (B) subunit of the heat labile toxin (Lt) of *Escherichia coli*. The *LtB* gene was synthesized to give optimum codon usage for corn and so maximize expression. Gene fusions were constructed to target LtB to various organelles in order to ensure protein stability. Using these approaches LtB was produced at a very high level in corn. As in *E. coli*, LtB produced in corn forms a pentamer that can bind to the GM1 receptor. Furthermore LtB pentamer stored in corn seed is much more resistant to heat than is the pure protein. As little as three doses of 5 micrograms of LtB presented in transgenic corn can induce strong serum and mucosal immune responses in mice. A level of protection to Lt holotoxin is also observed in mice fed corn that expresses *LtB*. This work demonstrates the potential of using corn seed as a delivery vehicle for subunit vaccines.

P-23

Monoclonal Antibodies from Corn Seed. JULIO BAEZ. Integrated Protein Technologies, 8355 Aero Drive, San Diego, CA 92123-1718.

Transgenic plants, already a proven technology in agriculture, can be used to produce therapeutic monoclonal antibodies. This presentation will focus on the first clinical results of a transgenic corn seed-produced injectable humanized monoclonal antibody and on the challenges, flexibility, robustness, and utility of this process. Information on the technology developed by Integrated Protein Technologies, a unit of Monsanto, for the production of Monoclonal Antibodies will be presented.

P-24

Use of Plant Roots for Biochemical Manufacturing. I. RASKIN. Biotech Center, Foran Hall, Cook College, Rutgers University., New Brunswick, N.J. 08901-8520. E-mail: raskin@aesop.rutgers.edu

Plant roots hold a large potential for bio-manufacturing and new lead discovery. Chemical and physical treatments, which mimic various stresses encountered by a plant, stimulate roots to produce arrays of new compounds. These elicited compounds may include valuable natural products and new pharmaceuticals. Plant roots can also be engineered to produce and continuously secrete several heterologous recombinant proteins. The yield of a recombinant protein produced via this so-called rhizosecretion technology may, over time may, exceed the weight of the plant producing it. The purification of proteins produced via rhizosecretion is a relatively simple task compared to tissue extraction methods.

P-25

Knowledge Generation and Transfer: The Case of Plant Biotechnology. GORDON C. RAUSSER. College of Natural Resources, University of California, Berkeley. E-mail: RAUSSER@NATURE.BERKELEY.EDU

In the generation and distribution of knowledge, distinctions are often drawn between science and technology. Science is often viewed as a non-market allocation mechanism where knowledge is treated as a pure public good. In contrast, technology is a market allocation mechanism where knowledge is treated as a private good, and where patents, copyrights, and trade secrets preserve property rights. In this context, the creation of knowledge, alternative paradigms for R & D, the critical role of Land Grant Universities and the design of public-private alliances are examined. The markets for knowledge in plant biotechnology are investigated, emphasizing recent developments in intellectual property, market liberalization, as well as market failures. Creating value with a focus on markets for technology is one thing, capturing value is quite another. Hence, the analysis turns on the commercialization process, competitive advantage and the role of complementary assets in capturing the value of knowledge. Finally, a number of concerns and challenges are presented about the potential future role of plant biotechnology, with sharply drawn future scenarios highlighting the impending evolution.

P-26

Biotechnology in the Developing World: Challenges and Opportunities. C. S. PRAKASH. Center for Plant Biotechnology Research, Dept of Agriculture, Tuskegee University, Tuskegee, AL 36088. Email: PRAKASH@TUSK.EDU>

Nearly a billion people go hungry every day, and there is a moral imperative for agricultural scientists to seek solutions to meet the food needs of the growing world. Genetic modification (GM) of crop plants holds considerable promise in enhancing food and nutritional security in the developing world. These countries face many constraints in harnessing biotechnology's potential including: problems with funding, access to technology and research infrastructure; limited scientific talent, concerns on the safety of GM food, lack of biosafety regulation, lack of robust intellectual property protection system, ideological opposition, concerns on the impact of GM crops on biodiversity and environment; and worries about trade and socio-economic factors. These factors must be creatively addressed to ensure expedited development of biotechnology in the developing world. Public sector institutions and international organizations such as CGIAR have major responsibilities in such development. Adequate biosafety regulations must be first developed to ensure development, testing and release of GM crops. Private sector can facilitate biotechnology development through offer of their core technologies on a 'royalty-free' basis for use on staple crops by public institutions. Efforts must be made to educate the policy makers, media, academics, farmers and the consumer in developing countries on the benefits of agricultural biotechnology and address their concerns. A constructive dialog with stakeholders will ensure responsible development of GM crops with minimal negative impact on the economy or the society.

P-27

Public Perceptions of Agricultural Biotechnology: Implications for Biologists. TJ HOBAN. Department of Sociology and Anthropology. NC State University, Raleigh, NC 27695-8107. E-mail: tom_hoban@ncsu.edu

After two decades of anticipation, the benefits of agricultural biotechnology are now becoming reality—at least for US farmers. The ultimate success of the biotechnology enterprise depends on how the public perceives and accepts the products. This presentation reviews public perceptions of agricultural biotechnology as measured by social science research conducted over the past decade. Survey results indicate the level of knowledge and acceptance of biotechnology expressed by consumers varies considerable around the world. Comparisons are made among people from different parts of the world and different demographic groups. Longitudinal analysis is also carried out to show trends over time. Results show that there is a urgent need for better communication between the scientific community and the lay public. This presentation provides a series of recommendations for how scientists can do a better job of communicating with the public about biotechnology and related topics. The outlook for future acceptance of biotechnology around the world is also discussed.

P-28

FDA Policy on Bioengineered Plant Foods. J.MCDONALD and J. Maryanski. U.S. Food and Drug Administration, Alameda, CA 94502. E-mail: jmcdonal@ora.fda.gov

Under the federal Food, Drug, and Cosmetic Act, companies have a legal obligation to ensure that foods for sale meet specific safety standards. In 1992, FDA published a policy whereby developers of bioengineered foods can consult with the agency to ensure that safety and regulatory requirements have been fully addressed. The policy also requires special labeling in certain circumstances, e.g., change in nutritional value or presence of an allergen not normally found in the conventionally-grown product. To date, biotechnology firms have completed consultation with FDA on more than 40 food products. While FDA is not aware of any reason to question the safety, quality, or any other attribute of currently marketed foods produced through bioengineering, it will consider any valid scientific information that suggests a reevaluation of agency policy. The outcome of a recent initiative to explain and seek public input about FDA's policy will be discussed.

T-1

Epithelial-Mesenchymal Interactions in Normal Tissue Homeostasis and Early Neoplastic Progression of Skin Equivalents. J.A. GARLICK. School of Dental Medicine, SUNY at Stony Brook, Stony Brook, NY 11794. E-mail: JONATHAN.GARLICK@SUNYSB.EDU

Homeostasis of stratified squamous epithelium requires complex interactions between epithelial and mesenchymal cells which determine epithelial differentiation, cell turnover, basement membrane formation and barrier function. *In vitro* models of human skin must mimic these features in order to effectively study the biology of this tissue. We will describe two skin equivalent models of human stratified epithelium which meet this criteria and facilitate the study of epithelial-mesenchymal interactions during normal tissue homeostasis and early neoplastic progression in this tissue. These two models are: 1—Organotypic Equivalents—which are generated entirely *in vitro* by growing human keratinocytes on a contracted collagen matrix (Organogenesis, Inc.) containing dermal fibroblasts and 2—Dermal-Epidermal Equivalents—which are generated by growing human keratinocytes on a de-epidermalized dermis (LifeCell, Inc.) which contains basement membrane but no fibroblasts. This dermis is grown on a contracted collagen matrix containing dermal fibroblasts which migrate into it. In combination, these tissue models have allowed us to manipulate two important variables in epithelial-mesenchymal interactions, namely, fibroblast-derived diffusible factors and basement membrane components. In this presentation, we will describe how we have used these tissue models to elucidate the influence of stromal elements on human stratified epithelial behavior and phenotype by: 1) elimination of viable fibroblasts, 2) addition of exogenous growth factors and 3) growing keratinocytes on varied basement membrane components. Understanding these events has allowed our laboratory to adapt these models to study the role of these factors in the development of premalignant lesions in stratified squamous epithelium. We will describe this premalignant tissue model and will demonstrate the response of potentially malignant keratinocytes to alterations in the stromal microenvironment. The significant role which these cell-cell interactions play in normal tissue homeostasis and the regulation of early cancer progression will be discussed.

T-2

Double Paracrine Regulation of Keratinocyte Growth and Differentiation by Mesenchymal Factors. N.E. FUSENIG, N. Maas-Szabowski, A. Szabowski, P. Angel. German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany. Email: N.FUSENIG@DKFZ-HEIDELBERG

Mesenchymal interactions are essential for epithelial growth and differentiation. For skin these interactions have been studied in organotypic cultures mimicking tissue physiology. Here an active interplay via diffusible factors produced by fibroblasts acting in a paracrine way on keratinocytes has been demonstrated. Moreover, keratinocytes actively regulate the expression of growth factors such as KGF and GM-CSF in fibroblasts by release of Il-1. The functional significance of this double paracrine pathway was documented by blocking and restoring both parts of this pathway. In order to discriminate regulatory mechanisms on growth and differentiation, signal transduction by Il-1 in fibroblasts was studied using mouse fibroblasts from AP-1 component knockout mice. Whereas c-jun-/- fibroblasts did not support keratinocyte growth, jun B-/- fibroblasts caused enhanced keratinocyte growth and differentiation. This was due to the lack of constitutive and Il-1 induced expression of KGF and GM-CSF by c-jun-/- cells and its enhanced expression by B-jun-/- cells. Moreover, growth-inhibited thin epithelia in c-jun-/-containing cultures could be partially rescued by KGF to a proliferative but less differentiating epithelium, whereas addition of both KGF and GM-CSF completely restored the wildtype phenotype. On the other hand, blocking antibodies to KGF reduced the jun B-/- phenotpye. Thus, keratinocyte proliferation and differentiation are regulated by fibroblasts via a fine tuning of the AP-1 transcription factor complex resulting in the balanced expression of KGF and GM-CSF as well as other cytokines.

T-3

Biology of Melanocytes and Melanoma in Human Skin Reconstructs. C. BERKING, F. Meier and M. Herlyn. The Wistar Institute, Philadelphia, PA 19104. Email: Berking@wistar.upenn.edu

In normal human skin the melanocytes are located in the basal layer of the epidermis. By transferring their pigment to the surrounding keratinocytes they form the epidermal melanin unit. Through cell-cell adhesion contacts mediated by E-cadherin keratinocytes control the growth and morphology of the melanocytes. During progression from a resting melanocyte to malignant melanoma this control is lost. A shift in the cadherin profile, i.e. upregulation of N-cadherin, enables communication with fibroblasts and endothelial cells, which may facilitate invasion and metastasis. Epidermal and dermal cell-cell interactions can be visualized in a three-dimensional skin reconstruct model, where melanocytes, nevus and melanoma cells derived from different stages of disease progression show the same growth pattern as in vivo. The transition from a metastasis-incompetent radial growth phase (RGP) melanoma to a metastasis-competent vertical growth phase (VGP) melanoma occurs when the beta3 integrin subunit of the alphaVbeta3 vitronectin receptor or the melanoma cell adhesion molecule (Mel-CAM) are overexpressed. The influence of specific growth factors in the skin microenvironment for tumor development and progression is being studied and the effects of UV irradiation are being tested in vivo after grafting to immunodeficient mice. Our human skin reconstruct model is ideal to investigate the contribution of individual genes to the biological behavior of melanocytes, nevi, and melanoma.

T-4

New Strategies for the Acceleration of the Drug Discovery Process, DAVID C. MYLES, Ph.D. Small Molecule Drug Discovery, Chiron Corporation, Emeryville, CA 94608. Email: david_myles@cc.chiron.com

The screening of combinatorial libraries in a high through put format has become a key component in the drug discovery process. This seminar will focus on strategies by which the time line for the discovery of lead structures from combinatorial libraries can be dramatically accelerated. This acceleration is achieved by a highly integrated combination of solid-phase library synthesis, in vitro biochemical assay, non-chemical deconvolution of hits from mixtures, and analytical chemistry.

T-5

Integrating Biology with New Drug Discovery Paradigm. KIA MOTESHAREI, Trega Biosciences, Inc. San Diego, CA 92121. E-mail: kmotesharei@trega.com

In today's modern age of drug discovery and with the recent advancements in genomics, combinatorial chemistry, and high-throughput screening, greater numbers of potential clinical candidates are being generated. With this surge in the number of potential clinical candidates has come an increased demand on biopharmaceutical screening methods. Among the factors determining the likelihood of potential clinical candidates becoming successful drugs are their pharmacokinetic and metabolism characteristics. The current *in vivo* pharmacokinetic and metabolism studies using animal models are too slow, inefficient, and cost ineffective to estimate compound bioavailability in humans. This increased demand associated in examining such potential clinical candidates makes the *in vitro* screenings of such candidates for pharmacokinetic and metabolism characteristics essential. The correlation of such *in vitro* data to actual performance in human is the following step in the decision making process in whether a compound is advanced through clinical development. Trega has developed a simulation modeling system that predicts pharmacokinetic characteristics in humans using *in vitro* data. Our computer simulation modeling and informatics system, IDEA™ (*In vitro* Determination for the Estimation of ADME), allows the prediction of oral drug absorption from *in vitro* parameters and hence creates a better selection process for advancement of drug candidates for development. The goal is to use this technology early in the discovery process to evaluate compounds for their clinical candidate potential with far less time and cost than traditional experimentally based methods. The modeling effort involves the use of actual *in vivo* pharmacokinetic performance of drug compounds in humans and animals.

T-7

Developing Target Cells Representative of Differentiated Cell Types Using Targeted Oncogenesis in Transgenic Mice. P.L. MELLON. Reproductive Medicine, UCSD, La Jolla, CA 92093-0674. E-mail: PMELLON@UCSD.EDU

Often the target cells for high throughput screening for newly developed drugs and hormones are rare cells found within complex tissues. Such cell types are not often represented by cell lines and when they are, they have often lost their differentiated characteristics. Using transgenic mice to target the expression of an oncogene to an individual cell type provides the power to develop clonal cultured cell lines to serve as model systems. Targeting expression of the SV40 T antigen oncogene using the regulatory regions of genes that are expressed only in the selected cell type allows for selective expression of the oncogene in a transgenic animal. For example, expressing the oncogene from pituitary-specific promoters produces cell lines that represent thyrotropes or gonadotropes and express differentiated markers such as luteinizing hormone, thyroid-stimulating hormone, activin and its receptor, estrogen receptor, progesterone receptor, and gonadotropin-releasing hormone receptor. These cells have served as ideal models for studies of pituitary physiology, endocrinology, and molecular biology. Using the regulatory region of the gonadotropin-releasing hormone (GnRH) gene to target the oncogene, we have produced specific tumors of the rare GnRH neurons of the hypothalamus. Clonal cell lines derived from hypothalamic tumors express GnRH mRNA and secrete accurately processed GnRH in the natural pulsatile pattern. They respond to a large number of neurotransmitters and modulators such as NMDA, GABA, endothelin, nitric oxide, prostaglandins, activin, histamine, FGF, norepinephrine and dopamine. This preservation of differentiated characteristics is likely due to coupling the expression of the oncogene to the specific regulatory region that selects for cells that maintain the differentiated state.

T-8

Genetic Programs of Hematopoietic Stem Cells and Microenvironments. I. R. LEMISCHKA, R. L. Phillips, N. Ivanova, H.-Y. Lin, R. Ernst, B. Brunk, G. C. Overton, and K. A. Moore.

The mammalian hematopoietic system originates from a rare population of stem cells which are multipotent in their differentiation potential and are endowed with self-renewal ability. A cellular and molecular characterization of the mechanisms which mediate the cell-fate choice between commitment/differentiation and self-renewal is key to understanding the functional regulation of the hematopoietic system both in normal and pathological states. As in all developmental systems, hematopoietic cell-fate decisions are governed by cell-intrinsic and microenvironmental mechanisms. As a foundation for unravelling the function of these mechanisms we have pursued strategies to define most if not all gene-products expressed prefentially in highly-purified undifferentiated stem cells isolated from the mouse and the human. A parallel effort is focused on the identification of a similarly broad panel of gene-products which is expressed preferentially in a stromal cell line which supports stem cells in vitro. Our basic strategy employs high-throughput sequence analysis of subtracted cDNA libraries enriched in gene-products prefentially expressed in stem cells or in the supportive stromal cell line. An emphasis is placed on sophisticated bioinformatic analysis of large sequence sets. To date, we have sequenced approximately 34,000 gene-products from ou rvarious efforts. A large proportion of these sequences represent novel gene-products, many of which have been placed into candidate stem cell regulatory categories based on the bioinformatic identification of their predicted protein product. Collectively, the data as well as the results of the analyses, have been placed in fully annotated, biological process oriented databases: Stem Cell Database (SCDb) and Strmal Cell Database (StroCDb). We have initiated functional studies for a number of particularly interesting gene-products, including novel transcriptional regulators and cell surface molecules. As a parallel approach, we have also initiated high-density array hybridization analyses on large panels of the characterized gene-products. The goal of these studies is to identify subsets of molecules whose expression fluctuates as a function of changes in the biological properties of the stem cells or the stromal cells. Our overall approach, integrating extensive sequencing, bioinformatic analyses, and array hybridization will allow the definition of regulatory pathways and networks collectively regulate fundamental aspects of stem cell biology.

T-9

Challenges and solutions to cell-based high-throughput screening and lead optimization. Thomas H. Large, PhD Sphinx Pharmaceuticals 20 TW Alexander Drive Research Triangle Park, NC 27709 E-mail: large_thomas_h@lilly.com

High-throughput screening (HTS) is established as a critical element in drug discovery and functional, cell-based assays are becoming the predominant HTS format. For rapid screening of compound libraries against drug targets, an important advantage of cell-based screening is the increased potential to identify broader structural classes of active compounds. For follow-up secondary efforts, cell-based assays can provide richer information on compound hits regarding target selectivity, mechanism of activity and additional cellular effects such as toxicity and proliferation. The current challenges for lead generation programs employing cell-based screens include: 1) Expedited assay development, e.g. the use of retroviral expression vectors and flow cytometry for obtaining desired cell lines, to keep pace with genomics efforts and target identification/validation. 2) Assay miniaturization, e.g. 384/1536 well formats and cell chip technologies, to allow increased screening throughput. 3) Development of automated, multi-probe assay formats, e.g. fluorescence sub-cellular microscopy, to provide high information content regarding compound activity. In addition to a focus on technological advances, a critical factor in successful HTS campaigns are personnel expert in cell and molecular biology, but capable of bridging the chemistry, automation and information technology fields. The reward to groups best able to develop advanced cell-based assays is a deeper understanding of compound activity and the identification of more and better drug leads.

V-1

Microgravity studies of cells and tissues. G. VUNJAK-NOVAKOVIC. Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge MA 02139 E-mail: gordana@mit.edu

Controlled microgravity studies of cells and tissues can improve our understanding of gravity sensing, transduction and response. This paper will discuss the scientific results and practical implications of two NASA-related projects: ground and space studies of microgravity tissue engineering (in collaboration with JSC—Houston), and the development of cell culture system for use aboard the International Space Station (in collaboration with ARC—Ames).Within the first project, a rotating bioreactor containing engineered cartilage was launched to Mir for a period of 4 months, while an identical bioreactor was operated as a ground control. Constructs from the two groups had markedly different structure and function (e.g. the compressive stiffness of constructs cultured on Earth was comparable to that of native cartilage and 3-fold higher than that of constructs cultured on Mir). These differences were consistent with previous reports that musculoskeletal tissues remodel in response to physical forces and are adversely affected by space flight. Within the second project, a fully automated system for cell and tissue culture is being developed for use both in microgravity (in the habitat holding rack) and in artificial gravity (in the space centrifuge). The unit can accomodate diverse biological specimens in up to 24 individual culture chambers, each being isolated from all other chambers and operated within a recirculation loop containing a gas exchanger, connections to media, additive and waste reservoirs, and a set of on-line optical sensors. Taken together, the results of these two projects demonstrate that the effects of microgravity on cells and tissues can be studied under controlled *in vitro* conditions.

V-4

Apoptosis in Human Peripheral Blood Lymphocytes in Modeled Microgravity. D. RISIN[1] and N. R. Pellis[2]. Cellular Biotechnology Program, [2]NASA/JSC and [1]Wyle Life Sciences, Houston, TX 77058. Email: drisin@ems.jsc.nasa.gov.

Spontaneous, activation- and radiation-induced programmed cell death (PCD) in human peripheral blood mononuclear cells (PBMC) were investigated in modeled microgravity (MMG) using the NASA rotating wall vessel (RWV) culture system. RWVs were developed at the Johnson Space Center and are commercially available from Synthecon, Inc. (Friendswood, TX). This culture system provides: 1) a state of continuous fall, 2) low shear environment and 3) randomization of gravity. MMG in the RWV inhibited radiation and activation induced apoptosis in PBMC, however, it did not affect the level of spontaneous apoptosis. Inhibition was observed in two types of experiments: 1) when PCD was induced by gamma-radiation of PBMC, and 2) when PCD in activated T cells was triggered by phytohaemagglutinin (PHA-M) or by phorbol myristate acetate (PMA)+ionomycin restimulation. Inhibition of apoptosis in activated T lymphocytes could not be attributed to changes in expression of Fas, FasL, bcl-2 or bax antigen. Adding of exogenous FasL abrogated the inhibitory effect of MMG, suggesting that MMG affects the interaction of membrane-bound Fas and FasL. The results indicate that MG might interfere with major physiological mechanisms involved in control of PCD in lymphoid cells and through this mechanisms contribute to the impairment of the immunity in space. (Supported by NRA OLMSA-02 and NSCORT NAG5-4072 grants).

V-5

Erythropoiesis in Simulated Microgravity. A.J. SYTKOWSKI, K.L Davis and J. Harrahy. Laboratory for Cell and Molecular Biology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215 E-mail: asytkows@caregroup.harvard.edu

The microgravity conditions experienced in space flight have been shown to have adverse effects on hematopoietic cells leading to anemia and reduced immune responsiveness. The cellular basis for these effects is unknown. We have now begun to investigate potential mechanisms responsible for the reduced erythropoiesis encountered in microgravity. We used the erythropoietin (Epo) responsive Rauscher murine erythroleukemia cells and erythropoietin-dependent BaF3-EpoR cells. We compared the growth and hormone responsiveness of these cells in unit gravity with the unique simulated microgravity environment of the NASA rotating wall vessel (RWV) bioreactor. Rauscher cells were inoculated into tissue culture flasks or dishes at 1xg or into the RWV in the absence or presence of Epo. At specified times thercafter ranging over a 4 day period, cell densities were determined and erythroid differentiation was quantified by determining the number of hemoglobin containing cells. We found a marked inhibitory effect of simulated microgravity on erythroid cell growth and differentiation. At both 1xg and in the simulated microgravity of the RWV, the cells grew at log phase for 72 hours. However, the growth rate in the RWV was significantly less than that at 1xg. The cells were equally viable under both conditions, and no increase in apoptotic cells in the RWV was detected. Epo induced differentiation under both culture conditions. However, the number of hemoglobin containing cells in the RWV was only half that observed at 1xg. Importantly, when cells were grown in simulated microgravity for 24 hours before addition of Epo, differentiation was inhibited virtually completely. Interestingly, Epo receptor binding studies of BaF3-EpoR cells revealed the appearance of a new class of receptors on cells grown in simulated microgravity. This was accompanied by an increase in steady-state Epo mRNA levels. These results suggest a profound effect of microgravity on erythropoiesis at the cellular level. This effect may be responsible in part for the anemia of space flight.

V-6

The Mechanics of Cell Regulation, Donald E. Ingber, M.D., Ph.D. Professor of Pathology, Harvard Medical School Departments of Pathology & Surgery, Children's Hospital Boston, MA 02115 ingber@a1.tch.harvard.edu

This lecture will focus on the role of cell structure and mechanics in the control of cell and tissue morphogenesis. Our work on angiogenesis (capillary development) has revealed that cell binding to extracellular matrix and associated mechanical deformation of cells (shape changes) can regulate morphogenesis in the presence of soluble growth factors by locally switching the cells between gene programs for growth, differentiation, apoptosis, contractility, and motility. By combining methods of molecular cell biology with engineering approaches, we have discovered that this mechanism involves mechanical stress-induced changes in cell, cytoskeletal, and nuclear structure as well as direct activation of integrin receptor signaling pathways. Cells also use integrins to sense mechanical stresses and to transmit these forces across the cell surface. These forces are then translated into changes in internal cell structure, biochemistry and gene expression via a tensegrity mechanism that integrates structure and function at all size scales in the hierarchy of cellular organization. We are currently carrying out studies using capillary endothelial cells, smooth muscle cells and fibroblasts to more precisely map out the series of molecular and biophysical events that mediate this form of mechanochemical transduction. Results of these studies should have widespread implications for control of tissue physiology and already have led to the development of a novel angiogenesis inhibitor (TNP-470) for the treatment of cancer. Some Relevant Publications: Wang N, Butler JP, Ingber DE. Mechanotransduction across the cell surface and through the cytoskeleton. Science 1993; 260:1124–1127. Ingber DE. Cellular Tensegrity: defining new rules of biological design that govern the cytoskeleton. J. Cell Sci. 1993; 104:613–627. Plopper G, McNamee H, Dike L, Bojanowski K, Ingber DE. Convergence of integrin and growth factor receptor signaling pathways within the focal adhesion complex. Mol. Biol. Cell 1995; 6:1369–1365. Chen CS, Mrksich M, Huang S, Whitesides G, Ingber DE. Geometric control of cell life and death. Science 1997; 276:1425–1428. Chicurel ME, Singer RH, Meyer C, Ingber DE. Integrin binding and mechanical tension induce movement of mRNA and ribosomes to focal adhesions. Nature 1998; 392:730–733. Ingber DE. The Architecture of Life. Scientific American Jan 1998; 278:48–57.

V-7

Cytoskeleton-Matrix Linkages in Motility: Effects of Force, Src and Position. M.P. SHEETZ*, D. Felsenfeld, T. Nishizaka, D. Raucher and C. Galbraith. Dep't of Cell Biology, Duke Medical Center, Durham, NC 27705. *Current address: Dep't of Biol. Sci., Columbia University, New York, NY 10027. E-mail: m.sheetz@cellbio.duke.edu

Cells generate defined forces on integrin-matrix contacts and respond to the forces that those contacts exert on them. A major hallmark of transformed cells is their ability to grow on soft agar, i.e. in the absence of force generated on matrix contacts. Interestingly, matrix contacts form preferentially within the first 0.5 micron from the leading edge of cell (fibronectin-coated beads bind 4X more in this region than 1–2 microns back on lamellipodial surfaces (Nishizaka et al., PNAS. 97:692–697(2000)). With rigid matrices, there was a force-dependent reinforcement of liganded integrin-cytoskeleton linkages in proportion to the force applied. Reinforcement and dissociation of linkages appears to be tyrosine phosphatase and kinase dependent, respectively. Tyrosine phosphatase inhibitors block reinforcement and the tyrosine kinase, Src, reverses reinforcement but only of vitronectin-avb3-cytoskeleton linkages (Felsenfeld et al., Nature Cell Biol. 1:200(1999)). To understand how cellular forces are generated and controlled, we have developed force sensors in silicon chips and have improved the laser tweezers measurements of isometric traction forces. Our studies show that traction forces are rearward in the front of all cells tested and switch to forward direction in the nuclear region (Galbraith and Sheetz, PNAS 94:9114–9118(1997) and J. Cell Biol. 147:1313–1323(1999)). Further, forces of the same level are generated on both dorsal and ventral surfaces, which validates the measurements of traction forces from the dorsal surface. We conclude that forces on matrix contacts are tightly controlled by cells through position and force-dependent linkages between matrix and force-generating elements of the cytoskeleton. We suggest that the transduction of force is predominantly at the site of application of force through elements of the integrin-cytoskeleton linkage and not through long-range sites as in a tensegrity model.

V-8

Laminar Flow Transduces an Atheroprotective Signal: Inhibiting TNF-alpha activation of JNK requires the ERK1/2 Pathway in Endothelial Cells. B.C. BERK & J. Surapisitchat. Center for Cardiovascular Research, University of Rochester, Rochester, NY 14642. BRADFORD_BERK@URMC.ROCHESTER.EDU

Atherosclerosis preferentially occurs in areas of turbulent flow and low fluid shear stress, while laminar flow and high shear stress are atheroprotective. Inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-alpha), have been shown to stimulate expression of endothelial cell (EC) genes that may promote atherosclerosis. TNF-alpha regulates gene expression in EC, in part, by stimulating Mitogen Activated Protein (MAP) kinases that phosphorylate transcription factors. We hypothesized that fluid shear stress modulates TNF-apha effects on EC by inhibiting TNF-alpha mediated activation of MAP kinases. To test this hypothesis, we determined the effects of steady laminar flow (shear stress = 12 dynes/cm2) on TNF-alpha stimulated activity of two MAP kinases: Extracellular signal Regulated Kinase (ERK1/2) and c-Jun N-terminal Kinase (JNK). Flow alone stimulated ERK1/2 activity, but decreased JNK activity compared to static controls. TNF-alpha (10 ng/mL) alone activated both ERK1/2 and JNK maximally at 15 min in human umbilical vein EC (HUVEC). Pre-exposing HUVEC for 10 min to flow inhibited TNF-alpha activation of JNK by 46%, but had no significant effect on ERK1/2 activation. Incubation of EC with PD98059, a specific mitogen-activated protein kinase kinase inhibitor, blocked the flow mediated inhibition of TNF-alpha activation of JNK. Flow mediated inhibition of JNK was unaffected by 0.1 mM L-nitro-alpharginine, 100 uM 8-bromo-cyclic GMP or by 100 uM 8-bromo-cyclic AMP. These findings indicate that fluid shear stress inhibits TNF-alpha mediated signaling events in HUVEC by a mechanism that is dependent on the activation of the ERK1/2 signaling pathway and independent of receptor activation, NO production or cyclic nucleotide generation. Inhibition of TNF-alpha signal transduction represents a novel mechanism by which steady laminar flow may exert atheroprotective effects on the endothelium.

V-9

Individualizing Cancer Chemotherapy by Tumor Histoculture. R.M. HOFFMAN [1,2], Y. Isobe[3], T. Kubota[4], K. Kubochi[3], T. Kinoshiita[3], T. Oishi[3], A. Shimada[3], M. Okuda[3], S. Ikeuchi[3], S. Shima[3], M.Kitajima[4], T. Furukawa[5], A. Moossa[2], S. Penman[6]. [1]AntiCancer, Inc., San Diego; [2]UCal, San Diego; [3]National Tokyo Medical Center, JAPAN;[4]Keio U, Tokyo, JAPAN; [5]Eiken Chem Co., Ltd., Tokyo; [6]MIT, Cambridge, MA. Email: all@anticancer.com

Cancers are highly individual in their response to chemotherapy but attempts to predict tumor response to drugs using *in vitro* cell culture have largely failed. A new *in vitro* technology, the Histoculture Drug Sensitivity Assay (HDRA), appears to have solved many previous problems. A prospective trial was conducted to determine whether effective agents for each individual patient could be distinguished by the HDRA. Tumor tissues from 30 patients with advanced gastric cancer and 19 with advanced colon cancer were placed in collagen-sponge-gel histoculture and treated with chemotherapeutic drugs and assayed for cell viability. Samples from 86% of gastric tumor cases and from 100% of colon tumor cases were successfully cultured and evaluated for chemosensitivity. Patient tumors were scored as sensitive in the HDRA if there was a response to at least one agent. HDRA-sensitive or HDRA-resistant patients were in all other regards clinically indistinguishable. Patients with HDRA-sensitive tumors were treated with the drugs scored as effective in the HDRA while the patients with HDRA-resistant tumors were treated by physician's choice. For patients with gastric cancer, the 50% survival of the HDRA-sensitive cases was 9.8 months compared to the 50% survival of 4.7 months for HDRA-resistant cases ($p = 0.02$). In colon cancer, the 50% survival in the HDRA-sensitive cases was 16.3 months compared to the 50% survival of 7.4 months for HDRA-resistant cases ($p = 0.02$). This is the first prospective, controlled trial demonstrating an *in vitro* assay that can distinguish those agents effective for survival of the individual cancer patient.

V-10

Stromal:epithelial interactions modulating invasion of human papillomavirus-transformed keratinocytes. JOEL M. PALEFSKY and M.A. Turner. Department of Laboratory Medicine, University of California, San Francisco, San Francisco, CA 94143. E mail: joelp@labmed.ucsf.edu

Human papillomavirus (HPV) is the etiologic agent of cervical and other anogenital squamous cell cancers (SCC). Cervical cancer is the most common cause of mortality among young women worldwide. The molecular mechanisms of progression from high-grade dysplasia to SCC and the role of stromal factors are poorly understood. Analyzing interactions between different cell types in this process is difficult in animal models. To model fibroblast:epithelial cell interactions in vitro we used the Matrigel artificial basement membrane invasion model. Fibroblast cell lines were established from a cervical SCC (cancer fibroblasts, CF) and from normal adjacent cervical tissues (normal fibroblasts, NF). CF and NF were added to the Matrigel. Their effects on invasion through the Matrigel of HPV-transformed foreskin keratinocytes (HK) or their primary parental keratinocytes (NK) were quantified using electron microscopy. In this model, HK by themselves were invasive but the NK were not. NF or its conditioned medium (CM) had no effect on invasion of either HK or NK. CF or its CM stimulated invasion of HK but not NK. This effect was abrogated with antibodies to basic fibroblast factor (bFGF). mRNA in situ hybridization of SCC confirmed specific expression of bFGF in stromal cells surrounding cancer nests. Our data suggest that there may be feedback loops mediated at least in part by bFGF between fibroblasts and keratinocytes and these effects are limited to CF and HK. Other factors studied in this model included the effect on invasion of alterations of keratinocyte proteinase expression and integrin expression associated with HPV-induced transformation. The Matrigel model, combined with confirmatory studies in SCC tissues is a useful approach to study complex cell interactions in cancer pathogenesis.

V-11

Human Myoepithelial Cells Exert Multiple Suppressive Effects on Breast Carcinoma Proliferation, Invasion and Angiogenesis. SANFORD H. BARSKY, Department of Pathology, UCLA School of Medicine, Los Angeles, CA 90024. Email: sbarsky@ucla.edu

Human Myoepithelial Cells Exert Multiple Suppressive Effects on Breast Carcinoma Proliferation, Invasion and Angiogenesis. SANFORD H. BARSKY, Department of Pathology, UCLA School of Medicine, Los Angeles, CA 90024. Email: sbarsky@ucla.edu

V-12

Synthetic Extracellular Matrices for Tissue Engineering. D.J. MOONEY. Depts. Biologic & Materials Sciences; Chemical Engineering, University of Michigan, Ann Arbor, MI 48109-1078 E-mail: mooneyd@umich.edu

Engineering tissues (e.g., muscle) by culturing cells on three dimensional biodegradable polymers is an exciting new approach to replace tissues and organs lost to disease or trauma. However, the cells within these tissues must exhibit the appropriate pattern of gene expression, and the tissues must develop with the appropriate structure if this approach is to be successful. The native extracellular matrix (ECM) of tissues aids both of these processes by providing a variety of chemical and mechanical signals to the forming tissue. We are attempting to create synthetic analogs to the ECM from biodegradable polymers that will similarly provide appropriate signals to the cells in the engineered tissues. We have determined that proper design of the mechanical properties and degradation rates of the synthetic matrices allows one to control the macroscopic structure of the engineered tissue. The gene expression of the cells in the tissue, and the tissue microstructure can be further regulated by controlling the mechanism of cell adhesion to the matrices, the release of growth factors from the matrices, and mechanical signals conveyed to the cells via their adhesion to the matrix. In summary, appropriate design of three-dimensional polymer matrices allows one to control the macrostructure and microstructure/function of engineered tissues, and this approach may hasten the development of clinically useful engineered tissues.

V-13

Bioreactor cultivation of engineered cartilage and cardiac muscle. G. VUNJAK-NOVAKOVIC. Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge MA 02139. E-mail: gordana@mit.edu

Tissue engineering has been motivated by the need to create functional biological substitutes of living tissues that can maintain, improve or restore tissue function. The approach discussed in this paper involves the use of primary or precursor cells (e.g. from cartilage, heart, bone marrow) which are seeded onto biodegradable, 3-dimensional polymer scaffolds and cultured in bioreactors. The resulting constructs can be used *in vitro*, for controlled studies of tissue development and function, or *in vivo*, for tissue repair. Construct structure (e.g. composition, morphology) and function (e.g. biosynthetic activity, mechanical or electrophysiological behavior, integrative potential) depend on the conditions an duration of bioreactor cultivation. The use of bioreactors generally permitted cultivation of larger, better organized engineered tissues than can be grown in conventional culture dishes. In addition, flow conditions within bioreactors can be utilized to modulate tissue formation by enhancing mass transfer (e.g. of oxygen, nutrients, metabolites) and by direct physical stimulation of the cells. Bioreactor cultivation of chondrogenic cells on polymer scaffolds resulted in large (1 cm in diameter, 6 mm thick) mechanically functional cartilaginous constructs. Likewise, neonatal rat heart cells cultured on polymer scaffolds in bioreactors formed cardiac constructs which contracted synchronously and contained electromechanically coupled cells expressing cardiac-specific tissue markers. The use of bioreactors for modulation of tissue structure and function by local environmental signals is discussed in more detail for engineered cartilage and cardiac muscle.

V-14

Current Clinical Trends and Changing Concepts in Tissue Engineering. ANTHONY ATALA, Laboratory for Tissue Engineering, Childrens Hospital and Harvard Medical School, Boston, MA 02115. E-mail: atala@a1.tch.harvard.edu

Research in the area of tissue engineering will be reviewed, including numerous technologies developed at the Laboratory for Cellular Therapeutics and Tissue Engineering at Children's Hospital and Harvard Medical School in Boston, some which are currently in Clinical Trials. Information regarding the creation of composite structures and the use of injectable tissue engineering materials will be presented. Future directions, including advances in gene therapy and fetal intervention, and how these are being combined with tissue engineering, will also be discussed.

V-15

Wild-Type FGFR2-IIIb/KGFR gene inhibits growth and induces differentiation/apoptosis in salivary gland tumors in vitro and in vivo. Y. ZHANG, M. KAN, W.L.MCKEEHAN, S.TORATANI and T. OKAMOTO. Molecular Oral Medicine & Maxillofacial Surg. 1, Hiroshima Univ. Faculty of Dentistry, Hiroshima 734–8553, Japan. E-mail:yanyan@ipc.hiroshima-u.ac.jp

We have previously reported that normal salivary gland epithelial cells and benign salivary gland tumors express exclusively KGFR/FGFR2-IIIb which recognizes stromal cell-derived KGF, and that in the process of malignant transformation of the tumors, the expression of KGFR/FGFR2-IIIb disappeared and an abnormal activation of FGFR1-IIIc gene expression which recognizes tumor-derived FGF-2 was observed. Thus, in this study, we have studied a potential role of wild-type KGFR/FGFR2-IIIb gene in growth, differentiation and apopatosis of human malignant salivary gland adenocarcinoma cell line HSY in vitro and in vivo. The growth of HSYR2-IIIb in serum-free medium was significantly decreased compared to that of HSYzeo. None of FGFs stimulated the growth of HSYR2-IIIb. TUNEL positive cells, DNA ladder formation and increase of CPP32/Caspase-3 activity were observed in HSYR2-IIIb. FGF-1 and FGF-2 stimulated the phosphorylation of both MEK1/2 and p38 MAPK in HSY and HSYzeo. In contrast, FGF-1, FGF-2 and KGF stimulated MEK1/2 phosphorylation but not P38 and JNK/SAPK in HSYR2-IIIb. Growth of HSYR2-IIIb tumors in athymic mice were dramatically decreased compared to that of HSYzeo. Some clones of HSYR2-IIIb lost their tumorigenicity. By histological examination, HSYR2-IIIb tumors exhibited differentiated morphology such as acinar-like and duct-like structure.KGFR gene therapy by electroporation completely cured HSY We have shown in this study that wild-type KGFR gene inhibits growth and induces differentiation and apoptosis in HSY cells in vitro and in vivo. These results strongly suggested the possibility that KGFR gene therapy might be a good alternative to cure salivary gland adenocarcinomas.

V-16

The Hepatocyte Growth Factor and Urokinase-type Plasminogen Activator in Lung Injury and Repair. K. Takahashi, T. Matsuoka. Fifth Department of Internal Medicine, Tokyo Medical University, Ibaraki, Japan. E-mail: kimikot@tokyo-med.ac.jp

Hepatocyte growth factor (HGF), initially identified as a potent mitogen for hepatocytes, has recently been suggested to be a pulmotrophic factor in vivo. Urokinase-type plasminogen activator (uPA) is known as an important protease, which converts plasminogen to plasmin for cell migration and activation of proenzymes or pro-peptides. Using cultured human pulmonary cells, the production and possible interaction of HGF and uPA was examined. Human lung fibroblasts and human lung microvascular endothelial cells (HLMECs) were isolated from normal regions of lungs of patients undergoing resection for solitary lung tumors. These cells, particularly HLMECs (10 fold in amount), produced and secreted HGF into the medium. The HGF antigen was also detected on the cell surfaces. HLMECs produced uPA and secreted it both to the luminal surface and to the basement membrane. IL-1 beta stimulated the HGF and uPA production of HLMECs in a dose dependent manner. HLMECs expressed the mRNA of c-met, the HGF receptor, suggesting the formation of HGF-receptor complex on the cell surfaces. Only in hyperoxic conditions, lung fibroblasts expressed the HGF mRNA. Although fibroblasts did not express c-met mRNA even when stimulated with hyperoxia, the HGF antigen was clearly detected in their extracellular matrix by using a conforcal-scanning microscopy. These results suggest that HGF may act as a mitogen for repair after lung injury, and uPA may play a role as pro-HGF activator in this microenvironment.

V-17

Autocrine growth factor and estrogen-independence in human breast cancer cells G. SERRERO, R.Lu and J. You. Dept. Pharmaceutical Sciences, University of maryland School of Pharmacy. Baltimore, MD 21201. E-mail:gserrero@rx.umaryland.edu

PC-Cell Derived Growth Factor (PCDGF) also known as epithelin/granulin precursor is a novel growth factor shown to stimulate the proliferation of mesenchymal and epithelial cells. In human breast cancer cells, PCDGF expression is stimulated by estradiol (E2) in estrogen receptor positive (ER^+) cells and is constitutively elevated in ER^- carcinomas. Experimental evidence will be provided here that PCDGF mediates E2 mitogenic effect in ER^+ cells. PCDGF overexpression leads to E2-independence and tamoxifen resistance. In ER^- cells, inhibition of PCDGF expression leads to inhibition of tumor formation. These data provide new information about a novel autocrine growth factor in human breast cancer.

V-18

Activin Plays an Important Role in Morphogenesis of Salivary Gland Epithelial Cells. M. Furue [1] and M. Asashima [2]. [1]Department of Biochemistry, Kanagawa Dental College, Yokosuka, Japan 238-8580. [2]Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan 153-0041. E-mail:mihofuru@kdcnet.ac.jp

Defining the mechanisms in morphogenesis leads to an understanding of the processes during regeneration. In salivary glands (SG), proper branching during morphogenesis is essential to maintain organ functions. This process appears to be tightly controlled by several growth factors, including hepatocyte growth factor (HGF), fibroblast growth factor (FGF) and activin. Since activin had been reported to play a critical role in mesoderm induction during early development, it has been considered that activin is likely to be involved in development and morphogenesis. Activin have been expressed in SG ductal epithelial cells. To study the role of activin in SG regeneration, we determined the effects of HGF, FGF, and activin on morphogenesis of epithelial RSMG-1 cells, which were derived from 10-week-old female rat submandibular gland. HGF intensely promoted RSMG-1 cells branching morphogenesis with forming lumina in collagen gel culture. FGF-1 promoted the cell proliferation, and branching morphogenesis synergistically with HGF. These two factors regulated activin mRNA expression in the cells. Activin was expressed in the cells forming ductal-like lumina, and its receptors localized at the branching point of the cell colonies in collagen gel. Exogenous activin inhibited the cell proliferation and regulated the branching morphogenesis of the cells. We conclude that activin may function in regeneration by regulating the growth and morphogenesis in SG.

V-20

INTERACTION BETWEEN HEMATOPOIETIC STEM CELLS AND ENDOTHELIAL CELLS. Toshio Suda and Nobuyuki Takakura, Department of Cell Differentiation, IMEG, Kumamoto University School of Medicine, Kumamoto, 860-0811,Japan e-mail:sudato@gpo.kumamoto-u.ac.jp

The formation of hematopoietic organs is dependent on the angiogenesis, indicating the existence of common progenitors, hemangioblasts which are able to differentiate into both hematopoietic stem cells (HSCs) and endothelial cells (ECs). We have shown that the first site where HSCs proliferate is omphalomesenteric artery (OA) besides para-aortic splanchnopleural region (P-Sp). HSCs form aggregates, adhere to ECs and express TIE2/TEK, c-Kit and Flk-1 receptor tyrosine kinases. Using the culture system of P-Sp explants from TIE2 deficient embryos, we showed that TIE2 on HSCs are critical for definitive hematopoiesis as well as development of vascular system. Stimulation of TIE2+ HSCs in OA by Angiopoietin-1 (Ang-1), a ligand for TIE2, promoted the cell adhesion to fibronectin through integrins. These HSCs remarkably proliferated in the presence of Ang-1 and SCF. Since Ang-1 is expressed on TIE2+ cells, autocrine loop of Ang-1 in self-renewing HSCs will be discussed. On the other hand, we also show that HSCs are essential for angiogenesis during embryogenesis. To investigate the role of HSCs in EC development, we analyzed AML1 deficient embryos, which lack definitive hematopoiesis. These embryos showed defective angiogenesis in the head and pericardium. P-Sp explant cultures on stromal cells did not generate definitive hematopoietic cells and showed defective angiogenesis in the AML1 null embryo. Disrupted angiogenesis in P-Sp cultures from AML1 null embryos was rescued by addition of HSCs or Ang1. HSCs, which express Ang1, directly promoted migration of ECs. These results indicate that hematopoiesis and angiogenesis are closely linked with each other.

V-21

Multilineal Blood Cell Differentiation in a Human Bone Marrow Model. J.H. DAVID WU and A. Mantalaris. Department of Chemical Engineering, University of Rochester, Department of Chemical Engineering, Rochester, NY 14627-0166. E-mail: davidwu@che.rochester.edu

Bone marrow is the hemopoietic tissue and a primary lymphoid organ. Its intricate, three-dimensional architecture facilitates cell-cell and cell-matrix interactions and provides a microenvironment that supports self-renewal and multilineal differentiation. Traditional in vitro culture systems (flask cultures) are limited by the absence of normal marrow spatial organization and cellular interactions with the extracellular matrix. They deviate from the three-dimensional marrow structure, inducing artificial lipogenesis and cell flattening, and produce only limited cell lineages. Our hypotheses are: 1) the marrow's function in promoting multilineal hemopoiesis depends on its three-dimensional structure and 2) this function can be (substantially) reproduced in vitro only if its three-dimensional structure is preserved. We have developed a novel human ex vivo bone marrow model that mimics bone marrow both structurally and functionally. By providing an artificial scaffolding, we were able to obtain a three-dimensional growth configuration with high cell density and intimate physical contact between hemopoietic and stromal cells. Characterization of the stroma developed demonstrated that an anatomical tissue-like structure was obtained. More importantly, the three-dimensional culture system supported multilineal hemopoiesis, including the development of myeloid, erythroid, and lymphoid lineages. In conclusion, the ability to engineer a structural and functional human bone marrow mimicry ex vivo provides a more physiologically meaningful approach for delineating the hemopoietic microenvironment, identifying the cellular and molecular signals important in hemopoiesis, and could potentially lead to novel bone marrow technologies.

V-22

Human Cytotoxic T Lymphocyte Culture and Clinical Application in Tumor Therapy. T. OHNO, RIKEN Cell Bank, The Institute of Physical and Chemical Research (RIKEN),Tsukuba Science City, 305-0074, Japan. E-mail: tad-ohno@rtc.riken.go.jp

Cytotoxic T lymphocytes (CTL) have higher cytotoxicity than the other known tumor-specific killer lymphocytes such as natural killer (NK) cells, lymphokine-activated killer (LAK) cells, and tumor-infiltrating lymphocytes (TIL). Therefore they have been considered to be useful for adoptive immunotherapy. If autologous CTL could be generated, they should be better than NK, LAK and TIL for adoptive immunotherapy of human tumors. To generate CTL from peripheral blood mononuclear cells (PBMC), we utilized the culture medium RHAM-alpha which has originally been formulated for serum-free culture of human lymphocytes. By combining this novel enriched medium supplemented with an interleukin cocktail of IL-1,−2,−4, and−6, autologous plasma of the PBMC donor, and optionally interferon-gamma, we could induce autologous CTL from PBMC by culture on cultured tumor cells, on minced tissue fragments, or on formalin-fixed cells of glioblastoma multiforme (GBM). A high rate of induction (9 of 10 cases) of human autologous CTL was achieved in vitro from PBMC of renal carcinoma patients by this culture sytem. Also, paraffin-embedded thin sections of the tissue containing renal cancer were useful as the tumor antigen for CTL induction. The cytotoxicity of CTL against the renal carcinoma cells was inhibited by antibodies against MHC-class I, CD8, and CD3. For clinical application, when the autologous CTL against malignant gliomas were injected 3–4 times into the primary-tumor-resected cavity via an Ommaya tube, reduction on MRI of the recurrent tumors was observed in the patients. In a GBM patient, the tumor volume (estimated, 130 cm^3) was rapidly reduced to one-third, although re-recurrence of the tumor followed 40 days later. Slight but apparent rapid reduction of the tumor volume was observed in other GBM patients and, in an anaplastic astrocytoma Grade III patient, complete reduction of the tumor was observed. The patient is surviving for more than one year. In a renal cancer patient bearing lymph-node metastated cancer, the autologous CTL reduced the lymph-node volume to less than one-third. These results suggest that adoptive immunotherapy with autologous CTL generated from blood cells will be clinically effective against malignant tumors.(Acknowledgement) This study was supported in part by a grant from the Special Promotion Fund of the Japanese Science and Technology Agency.

V-26

Involvement of MMP Proteinase Systems and Specific Collagen Cleavage in a Quantitative Angiogenesis Model. M.SEANDEL and J.P. Quigley. S.U.N.Y.Stony Brook and The Scripps Research Institute, La Jolla, CA 92037. E-mail: jquigley@scripps.edu.

Matrix metalloproteinases (MMPs) have been widely implicated in angiogenesis. However, it remains unclear at present what subsets of MMPs are required for angiogenesis and which substrate(s) must be cleaved *in vivo* by these enzymes for new blood vessels to grow. As a quantifiable angiogenesis model we have utilized the chorioallantoic membrane (CAM) of the chicken embryo. We have implanted fibrillar collagen gels containing angiogenic stimulators onto the CAM to determine: (1) the nature of the specific cell types that accompany the formation of new blood vessels in the collagen/CAM implants, (2) the identity of the MMPs that are expressed in the implants, (3) the dependence of angiogenesis on MMP activity, and (4) the possible requirement for specific cleavage and remodeling of the fibrillar collagen. Analysis of collagen gels undergoing vascularization in response to basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) revealed a complex cellular environment, which included endothelial cells, myofibroblasts, macrophages and heterophils all identified with specific morphologic and immunologic markers. To address the question of whether MMPs are required for angiogenesis, a synthetic MMP inhibitor (BB3103) and a natural inhibitor (N-TIMP-1) were added to the collagen gels. The stimulation of angiogenesis by bFGF and VEGF was reduced 40–70% in the presence of either of these inhibitors. Since MMP-2 (gelatinase A) has been implicated as a mediator of angiogenesis, we determined the levels of the 72 kD zymogen form and the 62 kD activated form found in collagen/CAM implants exhibiting high angiogenesis scores. We also determined that a newly cloned and characterized chicken MMP (ch MMP-X) is differentially expressed in the collagen/CAM implants coincident with the appearances of new blood vessels. Since remodeling of the extracellular matrix is hypothesized to be the primary function of MMPs, we examined whether degradation of the collagen implant was required for angiogenesis. To this end, we compared the magnitude of vascularization using wild type (WT) or mutant, collagenase-resistant type I collagen. Interestingly, angiogenesis in the presence of growth factors was substantially diminished using the collagenase-resistant substratum. We conclude that MMP/collagenase-mediated cleavage of the type I collagen matrix is necessary to allow angiogenesis to proceed to completion. To our knowledge, this represents the first direct demonstration of a requirement for specific collagen cleavage in growth factor-induced angiogenesis.

V-27

MMP-9 binds to a ligand induced cryptic site within b1 integrin: Role in angiogenesis and tumor growth. Peter C. Brooks, Ph.D. USC Keck School of Medicine. Department of Biochemistry and Molecular Biology. Norris Cancer Center/ Topping Tower Rm. 6409. 1441 Eastlake Ave Los Angeles CA 90033. pbrooks@hsc.usc.edu

The growth and spread of malignant tumors depends in part on the development of new blood vessels or angiogenesis. Thus, an in-depth understanding of the mechanisms that control neovascularization is of great importance. To this end, angiogenesis is thought to depend on the cooperative interactions between a wide variety of molecules including, growth factors and their receptors, cell adhesion molecules, proteinases and extracellular matrix (ECM) components. Previous studies have suggested that localized proteolytic remodeling of the ECM may facilitate angiogenesis. However, little is known concerning the mechansims that coordinate proteolytic activity at the cell surface. Here we provide evidence of a unique mechanisms by which localized proteolysis may be controlled by a novel association between a matrix metalloproteinase (MMP-9) and a b1 integrin. Our studies suggest that ligation of fibronectin by the a5b1 integrin may result in the exposure of a cryptic MMP-9 binding site within the b1 chain. Exposure of this cryptic site may facilitate localization of MMP-9 to the endothelial cell surface. Importantly, we have generated a monoclonal antibody (Mab FM155) that recognises a conserved amino acid sequence that may play a role in mediating MMP-9 / b1 integrin interactions. In fact, this unique Mab was shown to potently inhibit angiogenesis and tumor growth in mutiple animal models. Taken together, these findings suggest that MMP-9 / b1 interactions may play an important role in tumor angiogenesis.

V-28

Inhibition of Angiogenesis by Plasminogen Activator Inhibitor-1. DANIEL A. LAWRENCE[1], Steingrimur Stefansson[1], Grainne A. McMahon[1], Michael K.K. Wong[2], Eric Peticlerc[3], and Peter C. Brooks[3]. Department of Vascular Biology[1], J.H. Holland Laboratory, American Red Cross, Rockville, MD, Department of Medicine[2], University of Pittsburgh, Pittsburgh, PA, and Department of Molecular Biology[3], Fred Norris Cancer Center, University of Southern California, Los Angeles, CA. Lawrenced@usa.redcross.org

Plasminogen Activator Inhibitor-1 (PAI-1) is the primary inhibitor of urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) and as such, is thought to be integral in regulating tissue remodeling associated with angiogenesis. PAI-1 also inhibits $alpha_v beta_3$-mediated migration of smooth muscle cells on vitronectin containing matrices via a mechanism, which is independent of its antiproteolytic activity. To further characterize the role of PAI-1 in vascular cell migration, and hence its potential role in the regulation of angiogenesis, we have examined the ability of PAI-1 to regulate FGF-2-induced migration and proliferation of human umbilical vein endothelial cells (HUVEC) *in vitro*. In a wound repair model, PAI-1 inhibited the migration of HUVECs in a proteinase independent manner by approximately 50%, suggesting that, as with smooth muscle cells, PAI-1 blocks endothelial cell migration by blocking access of $alpha_v beta_3$ to vitronectin. Consistent with this, antibodies against vitronectin inhibited the migration to a similar extent. In contrast, FGF-2-induced proliferation of HUVECs was unaffected by PAI-1 addition. These data suggest that HUVEC migration on vitronectin is $alpha_v beta_3$-dependent and that, while the growth potential remains unaffected by PAI-1 addition, cell migration may be inhibited by PAI-1. *In vivo* angiogenesis assays demonstrated that PAI-1 also inhibited FGF-2-induced angiogenesis in both the chicken chorioallantoic membrane (CAM) assay and the Matrigel implant assay in mice. Similar to the *in vitro* cell migration results, antibodies directed against vitronectin also blocked angiogenesis in the CAM assay, providing further evidence that endothelial cell migration *in vivo* may be mediated by vitronectin. Immunohistochemical analysis of Matrigel implants demonstrated that implants treated with FGF-2 contained numerous highly branched vessels located throughout the implant. In contrast, Matrigel implants containing both FGF-2 and PAI-1 contained may fewer cells, which were not organized into vessel-like structures and were located around the periphery of the implant. Staining for von Willebrand factor demonstrated that these cells were endothelial cells. In addition, staining for proliferating cells at the boundary of the Matrigel implant with antibodies to PCNA did not show any significant difference between PAI-1-treated and untreated implants, indicating that, in the PAI-1-containing implants, the cells were proliferating normally, but were impeded in their migration into the implant by the presence of PAI-1. These results provide evidence that PAI-1 inhibits FGF-2-induced migration *in vivo* and *in vitro* and suggests that PAI-1 is not only an important antiproteolytic factor, but also is important for regulating the migration and hence angiogenesis potential of vascular cells *in vivo*.

V-29

Adventitious Agents in Source Materials: Cells and Growth Media. G.N. STACEY. National Institute for Biological Standards and Control, South Mimms, Herts., EN6 3QG, UK. Email: gstacey@nibsc.ac.uk

Cell cultures and biological reagents derived from animals are potential sources of microbial contamination and testing for mycoplasma, bacteria and fungi can and should be performed as a matter of routine. In general the use of cell culture and cell derived products does not appear to represent a serious virological hazard to laboratory workers. However, the occasional reports of cell lines contaminated with human pathogens indicate that there is no room for complacency. Since relatively little (if any) virus testing is performed on the majority of cell cultures used in research laboratories it is wise to treat all cell cultures as potentially infectious. Contamination with non-pathogenic viruses is also of concern since it may have serious consequences for the quality and relevance of scientific data from contaminated cells. A wide range of techniques and approaches can be applied in viral testing and, where cell cultures or biological products are intended for medical use, this may be a very demanding process. In research and diagnostic situations it may be possible to prioritize certain tests based on a variety of factors including species of origin, cohort of origin, tissue type and cell type. PCR and reverse transcriptase-PCR methods are available for relevant pathogenic viruses and potential contaminants in calf serum and trypsin. These techniques can be highly sensitive but test samples may contain components that will inhibit critical enzyme activities. Thus appropriate controls and standards should be utilized to validate each set of test data. New infectious agents are discovered periodically and *in vitro* culture offers opportunities for the generation of novel viruses. Thus, whilst much can be done to confirm our confidence in the safety of cell cultures, they should still be handled with caution under appropriate conditions of containment by trained staff.

V-31

DNA Profiling and Cross-Contamination of Human Cell Lines. J.R.W. MASTERS, J.A. Thomson and P.G. Debenham. University College London, London W1P 7PN, UK and LGC, Teddington TW11 0LY, UK. E-mail: J.Masters@ucl.ac.uk

Cell line cross-contamination is a long-standing problem and a frequent cause of scientific fraud, usually but not always accidental. Cross-contamination can be detected using various methods, including the analysis of enzyme and DNA polymorphisms, HLA typing and karyotyping. Until recently there was no standard inexpensive method which could be universally applied to give reproducible results in different laboratories. This goal is now achieveable using the single tandem repeat (STR) profiling methods developed principally for forensic applications. Using this approach, a number of polymorphic STR loci are amplified and the PCR products run simultaneously with size standards using automated fluorescent detection. The result is a simple numerical code corresponding to the lengths of the PCR products at each locus. The cost for each cell line is less than that of a bottle of fetal calf serum. This code could provide an international reference standard for each cell line. In order to test this hypothesis, the DNA profiles of over 250 human cell lines from various sources worldwide were analysed by STR profiling using the SGM primer set. Cancer cell lines showed frequent peak height imbalance, three alleles at many loci and excess homozygosity. All the known, as well as some unknown, examples of cross-contamination between human cell lines were detected. The method also identified related samples, hybrids, virally transformed cells and sublines that had been selected for drug resistance. If this method is applied internationally using an agreed set of STR primers, cross-contamination of human cell lines will be readily detectable. Publication of information derived from cross-contaminated cell lines could almost be eliminated if journal editors require evidence of cell line authentication by standardised DNA profiling.

V-32

Cell Line Authentication: Cross-Contaminations and Misidentifications. H.G. DREXLER. DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany. Email: hdr@dsmz.de

There are 3 major problems regarding continuous cell lines: (i) availability as most originators are reluctant to deposit cell lines in cell line banks; (ii) mycoplasma contamination (incidence of 20–30%); (iii) false cell lines which are misidentified/cross-contaminated. The risk of adventitious contamination and subsequent overgrowth of cell lines by unrelated cells is a recurring problem of cell line culture. This problem of intra/interspecies cross-contamination among human cell lines has been recognized for over 25 years; incidences of 17–35% have been reported. The most useful methods to detect human cell cross-contamination are DNA fingerprinting and cytogenetics, each complementing the other. Using this combination, we undertook 2 surveys on the incidence of intraspecies cross-contamination: (1) all types of human tumor cell lines obtained from the original source; (2) the specific subset of leukemia-lymphoma cell lines, obtained from both originators and secondary sources. Widespread high levels of cross-contaminants were uncovered in the first survey, affecting 45/252 (18%) cell lines supplied by 27 of 93 originators (29%). The most prolific cross-contaminants were classic tumor lines such as HeLa, SK-HEP-1, U-937 and HT-29 besides ECV304, the most cited human endothelial cell line. The second survey covered 315 leukemia-lymphoma cell lines (35 as duplicates from different sources): 41/212 (19%) cell lines from the original source and 15/112 (14%) from secondary sources were cross-contaminated (total 56/324, 17%). The false cell lines have been used in hundreds of potentially misleading reports, including as inappropriate tumor models and subclones masquerading as independent replicates. These findings indicate a grave and chronic problem demanding radical measures and strict control of cell line authentication, provenance and availability.

V-33

A Profile of the ATCC Human Cell Line Collection. A.S. DURKIN, Y. A. Reid, and R. J. Hay. American Type Culture Collection, Cell Biology Collection, 10801 University Boulevard, Manassas, VA 20110-2209 USA. E-mail: sdurkin@atcc.org

The ATCC employs a DNA fingerprinting component in its rigorous multipartite cell line authentication process. The DNA profiles are generated using a commercially available kit based on eight short tandem repeat (STR) loci. Additionally, the gender of the donor material is either verified or determined based on analysis of the amelogenin locus. An overview of the tips, tricks and pitfalls of this DNA fingerprinting program will be presented. Specifically addressed will be the issues of peak imbalances and genetic anomalies that are inherent when working with non-diploid cell lines, the utility of gender determination, cell line misidentification and the need for the community to adopt a universal, portable DNA fingerprinting system when authenticating cell lines.

W-1

Designer Crops for Better Nutrition in Developing Countries. ASIS DATTA. Jawarhal Nehru University, New Delhi 110067, INDIA.

The Green Revolution has so far helped to keep the rate of growth in food production above the population growth rate. The emerging Gene Revolution, by contrast, has given biological science a new dimension for improving crop productivity and quality. As we approach the end of the 20th century, we see a rapid transition from mendellan to molecular genetic applications in agriculture, medicine and industry. Between 1996 and 1998, in just eight countries, the area covered by new genetically improved transgenic plants (from 16.8 to 27.8 million hectares). The United States, Argentina, Brazil, and China have moved ahead quickly. Research in India has also shown that genetic modification can do immense good in agriculture and food security. This is the prime area in which biotechnology offers major inputs for healthier and more nutritious food. Millions of people throughout the world are undernourished and children are most affected. A recent UNICEF report on food and nutrition deficiencies in children describes this as a slight, invisible emergency with no outward sign of a problem. Every year over 6 million children under the age of 5 die worldwide and about 2.7 million of these children die alone in India. More than half of these deaths result from inadequate nutrition. The exploitation of genetic manipulation, development of new hybrids, screening of new genes for abiotic and biotic resistance, and developing planting material with desirable traits and genetic enhancement of all important crops will dominate the research agenda in the next century. Towards this end, we have achieved the cloning and sequencing of two novel genes, *OXDC* from *Collyhia velutips* and *AmAl* from *Amaranthus hypochondriacus*. We have developed efficient regeneration and compatible transformation protocols for potato, tomato, rice and *Lathyrus*. The efficacy of OXDC in improving the agronomic performance of plant has already been tested in transgenic tobacco and tomato. The results strongly suggested that transfer of OXDC gene to plants with high oxalate content reduce the nutritional stress. In addition, the bioassay data with the detached leaves showed that the transgenic plants are completely protected from the infection of *Sclerotinia*. The expression of the *AmAl* gene resulted in a striking increase in the growth and production of tubers in transgenic potato populations as also of the total protein content with an increase in most essential amino acids. The results well document, apart from successful nutritional improvement of potato tubers, the feasibility of genetically modifying other crop plants with novel seed protein composition. Besides India, there are a number of developing countries where the genetically improved crops are ready for field trials. Thus the aims and objectives are laudable and the tools are available. Certainly the 21st century would witness a major increase in new bioproducts generated through genetic engineering. It should therefore be possible to develop capabilities and programs so that Gene Revolution brings the goods for the mankind.

W-2

Transfer of Transgenic Rice for IRRI to Asian Countries for Evaluation and Breeding. S.K. DATTA. Plant Breeding, Genetics, and Biochemistry Division, International Rice Research Institute, MCPO Box 3127, 1271 Makati City, Philippines. Email: S.DATTA@CGIAR.ORG

Genetic engineering has improved crops by incorporating many novel genes. The genes used come from heterogeneous sources such as viruses, bacteria, plants, and animals. The constitutive and tissue-specific expression of selected genes is now in place. On average, a 20–30% yield loss caused by weeds and disease and insect attacks is common in rice. Durable resistance with crop management is an integral part of sustainable agriculture that could help in achieving food security in Asia. More rice needs to be produces using less land, water, and agrochemicals. In addition to plant protection, it is possible to achieve high grain filling, provitamin A rice, and a change in plant architecture. Intellectual property rights, biosafety, and material transfer agreements are critical steps that need more attention than before. IRRI has transferred transgenic rice to China and agreements have been prepared for India and other Asian countries to transfer the transgenic rice seeds that contain genes for resistance to stem borer, bacterial blight, and sheath blight. This work will benefit all stakeholders in sharing scientific outcomes and providing better acceptance of transgenic products. Improved rice breeding with the transgenic approach could play a key role in achieving food security in Asia. Such knowledge-based technology should be made available to poor countries.

W-3

The Relevance of Ag-Biotechnology for Developing Countries Revisited. A.F. KRATTIGER. Cornell University, 260 Emerson Hall, Ithaca, NY 14853. E-mail: afk3@cornell.edu

The rapid adoption of transgenic crops in industrialized countries is reviewed, together with major developments in the development of transgenics as may be applicable to developing countries. A brief review on the status of transgenic crops in developing countries is also reviewed. The presentation will address major issues that affect the transfer of biotechnology applications to developing countries, including the consolidation in the agri-biotech industry, donor investments and funding strategies, the roles of NGOs, international agreements such as the Convention on Biological Diversity and environmental issues.

W-4

Field Collection of Woody and Herbaceous Explants for In Vitro Culture. M.H. RENFROE, J.T. Hitt, P.J. McNicholas, and J.F. O'Hara. Department of Biology, James Madison University, Harrisonburg, VA 22807 E-mail: renfromh@jmu.edu

Germplasm conservation efforts may be augmented by plant tissue culture. Establishment of axenic cultures from field-collected materials depends upon elimination of the bacterial and fungal population on the explant surface, and maintaining the health of the explant prior to placement on a culture medium. Explants were collected from woody and herbaceous species, treated for microbial reduction and planted or stored for three days to simulate shipping prior to planting on culture medium. For woody plant species (*Betula pendula*, *Corylus avellana*), dormant buds were collected in the field, treated with ethanol and/or an anti-microbial solution, then held with or without a weak nutrient solution. In the laboratory, explants were treated with hypochlorite solution and some were placed in an anti-microbial solution prior to planting on culture medium. Best results were obtained by treating with ethanol, storing in an anti-microbial solution, and treating with hypochlorite prior to planting. For herbaceous plant species (*Portulaca oleracea*) collection and planting in the field was compared with holding and planting in the laboratory, or direct transport to the laboratory and planting. Best results were obtained with direct transport and sterilization in the laboratory. Direct planting in the field produced most contamination, even with anti-microbial agents in the medium. For field collection and delayed planting, a treatment in the field and laboratory with anti-microbial agents in the medium was most beneficial.

W-5

In Vitro Collection Techniques for Leaf and Bud Tissues. V.C. PENCE, B.L. Plair and J.R. Clark. Center for Research of Endangered Wildlife, Cincinnati Zoo and Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220. E-mail: vcpence@aol.com

In vitro collection techniques have been developed in this laboratory for broadly applied collection of leaf and bud tissues. These methods have been used to collect nonendangered plant tissues from Trinidad, Costa Rica, and the Cincinnati area; and endangered species from North Carolina, Florida and Utah. Tissues are surface disinfested with 70% ethanol after removal from the plant and transferred to small vials of sterile, semi-solid medium. For general collection, a Linsmaier and Skoog basal medium plus 3% sucrose, 0.22% Phytagel (Sigma), and 0.5 mg/L each of BAP and NAA has been used. Active benlate, at a concentration of 100 mg/L, has been included in the collection medium to control fungal contamination. In addition, either a drop of antibiotic solution is added on top of the explant shortly after collection, or 0.2–0.25 ml/L PPM is included in the medium. The percentage of cultures without visible contamination has ranged from 30–70%, depending on the collection site. Higher contamination rates generally occurred in cultures initiated at tropical sites, compared with temperate collections. Overall, a lower percentage of buds collected by IVC has continued growth in vitro compared with leaf tissue, but IVC of buds has been used successfully to collect tissues from several U.S. endangered species. Continued work should improve the technique, but even current methods for IVC offer a significant opportunity for the collection of plant germplasm when seeds are not available. Research supported in part by Institute of Museum and Library Services grant No. IC-70248-97.

W-6

Foraging Ascomycetes as Contaminants of Plant Tissue Cultures: Fungal Endophytes in Leaves and Stems. G.C. CARROLL. Department of Biology, University of Oregon, Eugene, OR 97403. Email: GCarroll@Oregon.uoregon.edu.

Terrestrial plants inhabit environments saturated with potentially invasive microbial cells. The fungi rank first among these, forming both pathogenic and mutualistic associations with living plants and serving as saprotrophic decomposers of dead plant remains. Thus, the ubiquitous occurrence of asymptomatic fungal infections in healthy plant tissues should come as no surprise. A few fungal endophytes, notably those in grasses, have been shown to act as protective mutualists against grazing mammals and insects by virtue of chemical defenses synthesized by the fungi. Many of these fungi are systemic in their host plants and may be transmitted through seed. In contrast most other plants are infected by fungi which form extremely limited infection domains, infections which become invasive only after injury, abscission, or contact with a nutrient rich media. These endophytes must reinfect young plant tissues as they are produced, and consequently they often sporulate profusely on natural substrates. Many of these fungi are latent stages of leaf-spotting Ascomycetes. Others consist of foraging states of wood and dung inhabiting Ascomycetes. While fungal infection domains may be limited in healthy plant tissues, fungal endophytes may grow rampantly when exposed to nutrient media. This is particularly true for endophytic *Xylaria* and *Phomopsis*, highly competitive wood-inhabiting fungi prevalent in the tropics. Such endophytes are certain to cause problems with in vitro plant tissue cultures made from material collected directly in the field. The incorporation of fungicides such as benomyl and anti-fungal antibiotics such as cyclosporin, either singly or in combination, might well alleviate these problems.

W-7

Disinfection of Field Collected Material Using Plant Preservatvie Mixture (PPM). A. GURI, H.Gazhali, R. Nick and D. Hartmann. Plant Cell Technology, Inc. Washington, DC 20036. E-mail GURI1@.EROLS.COM

PPM, an antimicrobial agent, was introduced at the end of 1996 into plant tissue culture specifically to prevent air or waterborne contamination. In the period of 1998–1999, the methodologies were improved to allow full to partial elimination of endogenous contamination and disinfection of in culture contaminated plant material. Explants with axillary buds of Pin Oak, Sycamore, Raspberry, Blackberry and Saka Palm, which normally cannot be disinfected with sodium hypochlorite or calcium hypochlorite, were stirred in a 50–100% PPM solution for 10–20 minutes. In the case of Pin Oak, exposing the explants (after 2 hours of water soak) to 50% PPM for 10 or 20 minutes and then transferring to medium without PPM yielded 84% and 92%, respectively, fungus-free explants. In another case, in culture contaminated orchid seedlings were decontaminated by shaking in a 2% PPM solution supplemented with 50 mg/l magnesium chloride for up to 48 hours. Consequently, 100% of those seedlings were bacteria- and fungus-free when placed on medium containing 0.2% PPM. PPM, if used correctly, will widen the range of species that can be collected from the field for in vitro propagation. Naturally, some species are more sensitive to PPM than others and, by the same token, some species are prone to higher endogenous contamination than others. An adjustment of the instructions by the users to meet their own specific need(s) is vital to the success of PPM.

W-10

Proteomics for crop improvement. GURU RAO, Pioneer Hi-Bred International, 7300 NW 62nd Avenue, Johnston, Iowa 50131-1004. E-mail: raog@phibred.com

Genome sequencing efforts on several plant species, including maize are underway. However, the sequence of the gene per se is less important than the nature of the genes expressed at a given time in any cell or tissue. Ultimately, it is the inter-relatedness of the genes and the gene products that are responsible for a given phenotypic trait. In this regard, high throughput technologies have become available in recent years to quantitatively measure transcript levels and establish global expression maps. However, it is becoming increasingly clear that since proteins define the business end of the gene expression pathway, a quantitative measure of expressed proteins may facilitate a more comprehensive assessment of the biology of a system. In this presentation I will provide an overview of Pioneer's research program in proteomics to discover genes for crop improvement.

W-11

Causal Connections: Programmed Cell Death, the Cell Cycle and Plant Disease. D. GILCHRIST, J. Lincoln, Z. Pan, P. Hoegger, C. Richael, and R. Bostock. CEPRAP University of California, Davis, CA 95616. Email: dggilchrist@ucdavis.edu.

Programmed cell death occurs in plants during development and in disease. Yet, existence of a genetic template and conserved signal transduction pathways directed to the selective elimination of certain cells to achieve cell homeostasis is a very recent revelation in modern biological research. Today, apoptosis, a form of programmed cell death (PCD), is accepted among animal biologists as a genetically controlled process that directs cells to die when they have completed an essential process, are in surplus, or have the potential to be deleterious to the organism. Results to be discussed indicate that there is considerable conservation at the cellular level of both the morphological and signaling features of apoptosis in plants. Both ceramide-linked signaling and transgenes from animal systems alter the response of plants to inducers of apoptotic-like PCD and to cellular pathogens of plants. Sphinganine analog mycotoxins (SAMs) are disease determinants in tomato and trigger cell death with morphological features of apoptosis. SAM-induced death can be blocked by ceramide analogs known to regulate the cell cycle, cell death and growth suppression in animal cells. These results suggest a functional linkage between cell cycle regulation, apoptosis and a novel lipid-based signaling pathway in plants during disease that also may be linked to the induction of apoptosis in plants as is reported in animal cells. While the existence and role of ceramide-based signaling in plant cells is virtually unknown, our ongoing parallel studies with animal and plant cells responding to diverse stimuli that affect apoptosis suggests a fruitful area of biochemical and genetic research in plants. In order to manipulate PCD processes in plants to assess their functional roles in disease will require linkage to specific genes and gene products that respond to a diverse set of signals transduced by the range of stimuli culminating in PCD.

W-12

Apoptosis-like cell death in a plant disease response. T.J. WOLPERT, W.C. Coffeen, and M.J. Curtis. Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331-2902. E-mail: wolpertt@bcc.orst.edu

Victoria blight of oats is caused by the fungus, *Cochliobolus victoriae*. The ability of the fungus to infect oats is dependent on its production of a selective phytotoxin called victorin. Only oats containing the dominant *Vb* gene are sensitive to victorin and consequently, susceptible to infection by the fungus. Victorin, a cyclized pentapeptide, has been shown to bind specifically to two proteins of the glycine decarboxylase complex (GDC), a multienzyme complex located in the mitochondrial matrix. GDC is involved in the photorespiratory cycle and inhibited by victorin both *in vivo* and *in vitro*. Photorespiration is initiated at the enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), and victorin has been found to induce a specific proteolytic cleavage of the Rubisco large subunit. In addition, victorin has been shown to induce internucleosomal DNA cleavage. Recent results indicate that victorin-induced Rubisco cleavage may involve a protease cascade with the participation of aspartate-specific proteases. Also, evidence suggests that victorin induces mitochondiral dysfunction. Thus, victorin-induced cell death appears to share some similarity with apoptosis. These results and their possible connection to victorin's interaction with the GDC will be discussed.

W-13

Maize D-type cyclin genes and their potential for improving transformation. YUMIN TAO, Keith Lowe, George Hoerster, Carolyn Gregory, Sheila Maddock, Dennis Bidney and Bill Gordon-Kamm. Pioneer Hi-Bred International, Inc., Johnston, IA 50010. E-mail: taoyumin@phibred.com

It has been speculated that for stable transformation to occur, the plant cell has to be actively dividing. Similar to other eukaryotes, cell division in higher plants is tightly regulated at the G1/S and G2/M boundaries. We have been searching for key genes involved in plant cell cycle regulation, and testing their potential to stimulate cell cycle progression and improve transformation. From Pioneer and DuPont's EST database, we have isolated a number of EST clones representing plant genes homologous to key cell cycle regulators from other organisms. Among them, a family of four maize D-type cyclin genes was studied in detail. Using approaches such as RACE and RT-PCR, we isolated full-length cDNAs. Sequence comparison of these full-length cDNAs showed that they share functional domains conserved among D-type cyclins, namely, the cyclin box and a LXCXE Rb-binding domain. Northern analyses indicated that expression patterns for these four D-type cyclins differ, suggesting they may have distinct roles in cell cycle regulation. Expression of a ZmCycD gene in a yeast mutant strain lacking all three G1 cyclins was able to rescue the cln yeast. Overexpression of two of the four ZmCycDs in maize Hi-II callus resulted in altered cell cycle profiles in flow cytrometric analyses. In addition, transformation frequency was increased when ZmCycD genes were over-expressed.

W-14

The Ins and Outs of the Plant Cell Cycle. P. CASTEELS. Department of Plant Genetics, VIB, Ghent Univ. E-mail: pecas@gengenp.rug.ac.be

The long-term goal of our research group is to understand the molecular regulation of the plant cell cycle and how it is affected following both intrinsic developmental signals and environmental factors. Indeed, a highly controlled cell division is essential for optimal growth and development in higher plants. Cell cycle progression is to a large extend steered by Cyclin-Dependent Kinase (CDK) protein complexes. CDK complexes consist of a catalytic subunit (CDK), a regulatory protein (cyclin) and optionally several other interacting proteins that affect the activity. These include, but are not limited to inhibitory proteins (CKI) and docking factors (CKS). CDK activity is additionally controlled by (de-)phosphorylation of specific residues and cell cycle phase dependent proteolysis of certain constituents. Transcript analysis during the cell cycle, two- and three-hybrid screens and biochemical purification of CDK/Cyclin complexes allowed to clarify some specific aspects of cell cycle proteins and their regulation during the cell cycle. We now start to understand how the cell cycle machinery is integrated with plant development and how external signals, such as environmental stress can put it on hold. For instance D-type cyclins appear to have a crucial role not only in driving the G1 phase of the cell cycle, but also in recruiting quiescent G0 cells into the cycle. Contrary, CKIs appear to have a role in arresting cell cycle progression. Recent data show that modulation of the cell cycle in transgenic plants often results in unexpected effects on growth, development and stress tolerance. This indicates that via an altered/modified cell cycle machinery we may improve plant architecture and resistance to stress.

W-15

Use of Maize LEC1 to Improve Transformation K. LOWE[1], S. Abbit[1], K. Glassman[1], C. Gregory[1], G. Hoerster[1], S. Rasco-Gaunt[2], X. Sun[1], P. Lazzeri[2] and W. Gordon-Kamm[1]. [1]Pioneer Hi-Bred. Intl. Inc. 7300 NW 62nd St. Johnston, IA 50131, [2]DuPont UK Ltd. Wheat Transformation Laboratory c/o Rothamsted Experimental Station Harpenden, Hertfordshire. AL5 2JQ UK. email loweks@phibred.com

Most cereal transformation techniques are dependent upon induction and proliferation of somatic embryos in culture. Traditionally, improvements in culture response have been made using media manipulations. We have taken a genetic approach to improve cereal tissue culture and transformation as an alternative to media manipulations. Other researchers have shown that ectopic expression of the Leafy Cotyledon1 gene (AtLEC1) in *Arabidopsis* triggers adventive embryo formation at low frequency from leaves. A maize LEC1 homolog (ZmLEC1) was identified with high similarity to the *Arabidopsis* LEC1 gene within the B-domain. The maize gene was placed into expression cassettes and evaluated in transformation experiments. A frame-shift version of this gene and a non-embryo specific maize HAP3 gene were also placed into maize expression cassettes for use as controls. Ectopic expression of the maize LEC1 greatly improved the recovery of transformants. A variety of inducible, tissue-specific and constitutive promoters were evaluated. Increased LEC1 expression produced concomitant increases in transformation frequencies and colony growth. The growth advantage imparted to LEC1 transformants allowed recovery of transformants without chemical selection at much higher frequencies than in controls. Parallel experiments were conducted in wheat. Transformation of the Zm LEC1 expression cassette into wheat, followed by selection for vigorous callus growth on medium without chemical selection produced high transformation frequencies with up to 61% of the visually selected calli carrying the transgene. To our knowledge this is the first example in plants where over-expression of an endogenous gene can be used for positive selection of transformants.

W-18

Genes that Control and Coordinate Embryogenesis in Higher Plants. J.J. HARADA, Section of Plant Biology, Division of Biological Sciences, University of California, Davis, CA 95616. Email: jjharada@ucdavis.edu

The Arabidopsis *LEAFY COTYLEDON (LEC)* genes play a central role in integrating several distinct aspects of embryogenesis. The *LEC* genes, *LEC1, LEC2*, and *FUSCA3*, are required a) to specify cotyledon identity, in part, by suppressing trichome formation on embryonic cotyledons, b) to allow embryos to become desiccation tolerant, c) to initiate and/or maintain the expression of a subset of seed protein genes during embryogenesis, d) to inhibit postgerminative development in developing embryos, and e) to suppress the embryogenic potential of the suspensor during early embryogenesis. The *LEC* genes are unique among other known embryonic regulators in that they function during both the early morphogenesis and late maturation phases of embryo development. We have cloned the *LEC1* gene and shown that it is homologous to a conserved eukaryotic transcription factor. Our studies suggest that *LEC1* plays a critical role in embryogenesis by establishing an environment that allows embryo development to occur. In this talk, I will discuss recent work from my lab to dissect *LEC1* function and to identify other *LEC* genes.

W-19

The Use of Phosphomannose Isomerase (*pmi*) as an Efficient Selectable Marker for Monocot and Dicot Transformation. J.N. REED, Y.F. Chang, J.L. Dawson, E.M. Dunder, G. Hansen, K.L. Launis, J.L. Suttie, X.H. Wang, A.R. Wenck, M.S. Wright. Novartis Agribusiness Biotechnology Research, Inc., 3054 Cornwallis Drive, Research Triangle Park, NC 27709. E-mail: janet.reed@nabri.novartis.com.

For genetic transformation of plants, most selection systems utilize antibiotics or herbicides. A selectable marker gene involved in simple sugar metabolism may be preferable in cases where these types of genes are not appropriate. The selectable marker, phosphomannose isomerase (*pmi*), encoded by the *manA* gene from *E. coli*, has proven to be efficient for transgenic plant production in a number of crop species using either microprojectile bombardment or *Agrobacterium* transformation. Phosphomannose isomerase converts mannose-6-phosphate, a non-usable source of carbon, into fructose-6-phosphate, a simple sugar that can be metabolized by plant cells. With the *pmi* system, selection occurs when mannose is incorporated into the selection medium replacing the usual carbon source, most often sucrose. The *pmi* selection system has been optimized for several genotypes of various crops with almost no escapes and with co-transformation frequencies as high as 94%, depending on the accompanying gene of interest. Transformation frequencies as high as 73% for maize and 39% for wheat using Biolistics(r) have been obtained with a mean of 50% and 18%, for maize and wheat, respectively. Transformation frequencies for maize using *pmi* and *Agrobacterium* transformation are as high as 32% with a mean of 10%. Molecular and expression analyses showed that the *pmi* gene was present, functional, and transmitted to progeny in a Medelian fashion. Once the *pmi* system is optimized for a particular genotype or transformation method, frequency of escapes is low while co-transformation and self-fertility of T0 plants are high. Additionally, transgenic plants from other crop species were recovered successfully using mannose selection.

W-20

Plant Selection Principle based on Xylose Isomerase. A. HALDRUP[1] and F. T. Okkels[2]. Danisco Biotechnology, Langebrogade 1, P.O. box 17, 1001 Copenhagen K, Denmark, [1] Plant Biochemistry Laboratory, Department of Plant Biology, The Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Copenhagen, Denmark, [2]Medi-Cult a/s, Moellehaven 12, DK-4040 Jyllinge. Email: anna@kvl.dk

The xylose isomerase genes (*xyl*A) from *Thermoanaerobacterium thermosulfurogenes* and *Streptomyces rubiginosus* were introduced and expressed in three plant species (potato, tobacco, and tomato) and transgenic plants have been selected on xylose-containing medium. The xylose isomerase gene was transferred to explants of the target plant by *Agrobacterium*-mediated transformation. The xylose isomerase gene was expressed using the enhanced cauliflower mosaic virus (CaMV) 35S promoter and the The xylose isomerase genes (*xyl*A) from *Thermoanaerobacterium thermosulfurogenes* and *Streptomyces rubiginosus* were introduced and expressed in three plant species (potato, tobacco, and tomato) and transgenic plants have been selected on xylose-containing medium. The xylose isomerase gene was transferred to explants of the target plant by *Agrobacterium*-mediated transformation. The xylose isomerase gene was expressed using the enhanced cauliflower mosaic virus (CaMV) 35S promoter and the W' translation enhancer sequence from tobacco mosaic virus. In potato and tomato, the xylose isomerase selection was more efficient than the established kanamycin selection. The level of enzyme activity in the regenrated transgenic plants selected on xylose was 5- to 25-fold higher than the enzyme activity in control plants. The xylose isomerase system enables the transgenic cells to utilize xylose as a carbohydrate source. It is an example of a positive selection system because transgenic cells proliferate while non-transgenic cells are starved but still survive. This contracts to antibiotic or herbicide resistance based systems where transgenic cells survive on a selective medium but non-transgenic cells are killed. The results give access to a new selection method, which is devoid of the disadvantages of antibiotic or herbicide selection, but depends on an enzyme that is generally recognized as safe for use in the starch industry and which is already being widely utilized in specific food processes.

W-21

MAT (Multi-Auto-Transformation) Vector System, "The Oncogenes of Agrobacterium Are Used as Positive Markers for Regeneration and Selection of Marker-Free Transgenic Plants." H. EBINUMA, K. Sugita, E. Matsunaga S. Endo, K. Yamada and A. Kawaoka. Central Research Laboratory, Nippon Paper Industries CO., LTD., 5-21-1, Oji, Kita-ku, Tokyo 114-0002, Japan. Email: LDW06374@nifty.ne.jp, 0880080@fr.nipponpaper.co.jp

Agrobacteria develop crown galls and hairy roots in a wide range of plant species. During infection, the growth-inducing genes, which are also called oncogenes, are transferred and integrated into the plant genome, and thus induce cell proliferation and organ differentiation through internal manipulation of plant hormone levels. Of the current transformation methods, we have exogenously applied plant growth regulators to induce regeneration and proliferation of transgenic tissues. Alternatively, oncogenes have not been used to induce proliferation and differentiation of transgenic cells because the regenerated transgenic plants exhibit seriously abnormal phenotypes. MATVS is designed to remove the oncogenes from transgenic plants by using the yeast site-specific recombination system R/RS and to recover the normal phenotype. This system enables us to use oncogenes as a tool for transformation of many recalcitrant plant species, including fruit trees and forest trees. We report here two kinds of MATVS: (1) the cytokinin type, in which the isopentenyl transferase (ipt) gene of A. tumefaciens PO22 is used as a selectable marker for regenerating transgenic cells and selecting marker-free transgenic plants. (2) the auxin type, in which the 7.6-kb EcoRI fragment containing the rol A, B, C genes of A. rhizogenes NIAES1724 is used. And we discuss the potential advantages of MATVS including: (1) improvement of transformation efficiency using oncogenes and (2) removal of marker genes without sexual crossings.

W-22

Seeing the Light: Utilization of GFP as a Visual Screenable Marker for Plant Transformation. H.F. KAEPPLER and A.R. Carlson. Dept. of Agronomy, University of Wisconsin,1575 Linden Drive,Madison, WI 53706. Email: hfkaeppl@facstaff.wisc.edu

The most widely utilized selectable markers for plant transformation have been herbicide and antibiotic resistance genes. New markers need to be developed, however, to improve the efficiency and flexibility of plant transformation, and to overcome drawbacks sometimes associated with use of resistance-based markers. Investigations were carried out to determine if green fluorescent protein (gfp) could be utilized alone as a visual screenable marker to produce stably transformed plants. Advantages of using gfp as a marker include visualization of transgene expression at any time without sample disruption, or addition of substrates, cofactors or selective agents. Additionally, selection media optimization studies are obviated, and inadvertent transfer of gfp to wild, weedy relatives of transgenic plants would not impart a selective advantage. Using the simple protocol described below, over 150 independent transgenic oat lines have been produced using only visual screening for expression of gfp. In our protocol, a red-shifted, codon-optimized, synthetic gfp gene under the control of the maize ubiquitin promoter and intron 1 is delivered into embryogenic oat callus via microprojectile bombardment. Cell clusters (0.5–2mm) expressing gfp are visually identified using epifluorescence microscopy and physically isolated approximately 3 weeks postbombardment. Fine-tipped forceps are used to separate and transfer glowing sectors to fresh culture medium every two weeks. Subsamples of gfp expressing cell lines are placed on regeneration medium about 9–11 weeks postbombardment. Fertile, gfp-expressing plants are regenerated and most produce gfp-expressing progeny in Mendelian ratios. Autofluorescence is not a problem in gfp-based selection of oat cells, however presence of autofluorescence in cell cultures of other cereal species has been observed and may reduce selection ability and efficiency. Cell culture growth rate and pattern may also influence the ability to perform gfp-based selection in some systems.

I-1001

Design of an Efficient Medium for Insect Cell Growth and Recombinant Protein Production. L. IKONOMOU[1], G. Bastin[2], Y.-J. Schneider[3], and S.N. Agathos[1]. [1]Unit of Bioengineering, Place Croix du Sud 2/19, [2]Centre for Systems Engineering and Applied Mechanics, Av. George Lemaître 4 and [3]Laboratory of Cellular Biochemistry, Place L. Pasteur 1, Catholic University of Louvain, B-1348, Louvain-la-Neuve, Belgium. E-mail: ikonomou@gebi.ucl.ac.be

There is an increasing demand for serum-free formulations in the field of animal cell culture, including insect cell culture and the baculovirus expression system. Nevertheless, commercially available media are often quite expensive and cell line specific. Furthermore, their composition is most often confidential, being in this way inappropriate for metabolic studies of insect cells. We report here the design of a serum-free medium suitable for the growth and recombinant protein production of the widely used insect cell lines, Sf9 and High-Five™. Initially, a variety of hydrolysates was screened by fractional factorial experiments for both cell lines, using IPL-41 as the basal medium. These included hydrolysates like yeastolate, commonly used in insect cell culture as well as other hydrolysates of animal and plant origin. Afterwards, a complete factorial design was employed in order to find the optimal concentrations of the selected supplements and to identify potential interactions. Furthermore, the concentration of specific amino acids like glutamine and asparagine was optimized to render it more efficient and to reduce the usually large ammonia concentrations (30–40 mM) found in batch cultures of High-Five cells. An increased concentration of lipids (a 400X dilution instead of the commonly employed 1000X) resulted in better growth. Yeastolate ultrafiltrate was shown to be indispensable for cell growth for both Sf9 and High-Five cell lines and it was added at a concentration of 0.6%. Addition of 0.5% Primatone RL further increased maximum cell concentration. The new medium can support high cell densities in batch culture ($4–5\times10^6$ and $5–6\times10^6$ cells/ml for High-Five and Sf9 cells, respectively) and high recombinant protein production. Surprisingly, lactate is not produced under oxygen excess by either of the cell lines, in contradiction to previously reported values (10–20 mM) for High-Five cells. Furthermore, its cost is about one third of that of most commercially available media. Overall, our serum-free medium emerges as an attractive alternative for inset cell culture, especially for modes of operation like perfusion where large quantities are needed.

I-1002

Comparison of Immobilization Methods for Insect Cells. L. IKONOMOU[1], J.-C. Drugmand[1], G. Bastin[2], Y.-J. Schneider[3], and S.N. Agathos[1]. [1]Unit of Bioengineering, Place Croix du Sud 2/19, [2]Centre for Systems Engineering and Applied Mechanics, Av. George Lemaître 4 and [3]Laboratory of Cellular Biochemistry, Place L. Pasteur 1, Catholic University of Louvain, B-1348, Louvain-la-Neuve, Belgium. E-mail: ikonomou@gebi.ucl.ac.be

Production of recombinant proteins by insect cells at high cell densities infected with recombinant baculoviruses has been shown to decrease dramatically due to depletion of key nutrients rather than contact inhibition. However, very few studies have been performed on the growth of immobilised insect cells either in fixed bed or in microcarrier systems, which are both high-density systems. We carried out a systematic study of the immobilization of insect cells (High-Five™ and Sf9 cell lines) in microcarrier cultures. The various microcarriers studied, included the Fibra-Cel disks (New Brunswick Scientific), never used before in suspension with insect cells, Cytodex 1 (Pharmacia), and glass microcarrier beads (Sigma). In the case of compact microcarriers (Cytodex I and glass beads) cells detached very rapidly from the microcarriers and continued to grow normally in the medium. We did not study baculovirus infection with this type of microcarriers as it was impossible to immobilize the cells prior to infection. Regarding the Fibra-Cel disks, we performed growth and infection studies at a disk concentration of 10 g/l having previously established the best inoculation densities for the two cell lines ($2.5\text{x}10^7$ and $3.75\text{x}10^7$ cells/g disks for High-Five and Sf9 cells, respectively). We were able to keep fed-batch cultures of high viability for over 400 hours reaching a cell concentration of $40\text{x}10^7$ cells/g disks in the case of Sf9 cells. Infection of Sf9 cells on the disks with a MOI of 2 resulted in similar maximum total beta-galactosidase titers with those obtained by free cell suspension cultures (586 IU/ml and 475 IU/ml, respectively). On the contrary, High-Five cells on Fibra-Cel produced less than half the beta-galactosidase compared to free suspension cultures (215 IU/ml and 586 IU/ml, respectively). It was not clear whether the low titer was due to nutrient limitation (other than glucose and glutamine) or to the significant accumulation of lactate (18 mM). The time span of protein production in the case of Fibra-Cel disks and for both cell lines was longer suggesting a non-synchronous infection. Similar studies are in progress with insect cells immobilized on Cultisphere G porous microcarriers (Percell Biolytica) as well as in a glass column on materials including Fibra-Cel disks, Dacron fibers and polyurethane (fixed bed configuration) or Cytoline microcarriers (air-lift configuration).

I-1003

Expression of Recombinant Endostatin in Stably Transformed *Drosophila melanogaster* S2 Cells. Jong Hwa Park, Kyung Hwa Chang, Jong Min Lee, In Sook Hwang and IN SIK CHUNG. Dept. of Genetic Eng., Kyung Hee University, Suwon, Korea, 449–701. Email: ischung@nms.kyunghee.ac.kr

Endostatin is a specific inhibitor of endothelial cell proliferation and angiogenesis. Systemic administration of purified recombinant endostatin has been shown to inhibit the growth of established tumors and metastases *in vivo*. Endostatin does not induce acquired drug resistance after several treatment cycles. In this study, we describes expression of recombinant endostatin from stably transformed *Drosophila melanogaster* S2 cells. Recombinant plasmids containing a cDNA coding mouse endostatin were transfected and stably expressed in *D. melanogaster* S2 cells. Stably transformed polyclonal cell populations expressing recombinant endostatin were isolated 4 weeks of selection with hygromycin B. Recombinant endostatin expressed in the stably transformed S2 cells under the influence of the *Drosophila* BiP protein signal sequence was secreted into the medium. Recombinant endostatin was also purified to homogeneity using a simple one-step Ni^{2+} affinity fractionation method. Purified recombinant endostatin inhibited endothelial cell proliferation in a dose-dependent manner. The stably transformed S2 cells produced 18 mg recombinant endostatin/L at 7 day post-induction with 5 mM $CdCl_2$. We are currently investigating the effect of bcl-2 gene on recombinant endostatin expression from *D. melanogaster* S2 cells.

I-1004

Gene Transfer to Cells of the Eastern Oyster. J.T. BUCHANAN[1], T. Cheng[2], J.F. La Peyre[2], R.K. Cooper[2], and T.R. Tiersch[3]. [1]Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803 USA. [2]Department of Veterinary Science and [3]Aquaculture Research Station, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803 USA. E-mail: jbuchanan@agctr.lsu.edu

Cell culture serves as an effective model for in vivo work. Research on gene transfer in eastern oysters is currently underway, and the use of cell cultures for screening promoters and genes for use in vivo is desirable. As a cell line from bivalve molluscs has not been established, it is necessary to work with primary cultures of oyster cells. Ventricle cells from *Crassostrea virginica* were isolated and conditions for optimal gene transfer and gene expression were investigated. At present we have examined several transfection reagents (SuperFect, Effectene, DoTap, and Lipofectamine Plus) for toxicity to cells and efficient gene transfer. We found increased toxicity to cells for transfection reagents combined with DNA compared to transfection reagents or DNA alone ($P < 0.05$). We screened several promoters (*C. gigas* actin, *Drosophila* actin, *Drosophila* heat shock, *Biomphalaria* heat shock, and SV40) for efficient gene expression. Expression of the reporter gene luciferase was detected and levels of expression found to vary with the promoter used, the transfection reagent used, and the concentration of DNA and transfection reagent. Gene expression also varied over time, with higher levels of expression observed within 12 h of transfection. From this preliminary work, the highest levels of expression were detected using the *Biomphalaria* heat shock promoter after heat shock at 40°C for 1 h.

I-1005

Pantropic retroviral vectors mediate foreign gene expression in shrimp (*Penaeus stylirostris*). H.SHIKE[1], C. Shimizu[1], K.R. Klimpel[2], J.C. Burns[1]. [1]Dept. of Pediatrics, UCSD School of Medicine, La Jolla, CA 92093-0830; [2] Super Shrimp Group Inc., 1545 Tidelands Ave., National City, CA 91950. hshike@ucsd.edu

The lack of a shrimp cell line has seriously hampered *in vitro* studies of shrimp pathogens, particularly viruses. Introduction and expression of immortalizing oncogenes is a standard approach to create a stable cell line, but no system currently exists for transduction of shrimp cells. To test pantropic retroviral vectors pseudotyped with the VSV-G protein as transducing agents for shrimp cells, we infected primary cultured cells derived from the lymphoid organ (Oka organ) and ovaries of 60–70 g adult shrimp (*P. stylirostris*). Cells were cultured in 2X L-15 medium supplemented with 20% FCS and antibiotics (final: 670 mOsm). Cultures were infected with either infectious retroviral vector (LLRNL: Moloney murine leukemia virus (MoMLV) long terminal repeat (LTR)-luciferase-Rous sarcoma virus (RSV) LTR-neomycin phosphotransferase-LTR) or non-infectious bald vector (lacking an envelope glycoprotein) at 0.5–1.0 h post-initiation of the cultures. After 72 h, cell lysates were prepared for luciferase and total cellular protein assays. Luciferase expression was consistently documented in LLRNL-infected Oka organ and ovary cultures but in none of the mock-infected or bald virus-exposed cultures. Infection with vectors expressing luciferase from different promoters (MoMLV LTR, RSV LTR, *Drosophila* heat shock 70 promoter, or baculovirus IE-1 promoter) demonstrated the highest levels of luciferase expression from the MoMLV LTR. Taken together, these experiments demonstrate that pantropic retroviral vectors are a suitable tool for the introduction and expression of foreign DNA in shrimp cells. Experiments are in progress to use these pantropic vectors to express oncogenes in shrimp cells to create an immortalized cell line.

I-1006

Infection of primary cultured cells from two oyster species by pantropic retroviral vectors V. BOULO[1], J. D. Moore[2], C. Shimizu[3], C. S. Friedman[2], and J.C. Burns[3]. [1]DRIM, IFREMER-CNRS, Univ. Montpellier II, 34095 Montpellier, France; [2]Bodega Marine Laboratory, Univ. of California Davis, Bodega Bay, CA 94923; [3]Univ. of California San Diego, 9500 Gilman Dr., La Jolla, CA 92093. Email: Viviane.Boulo@ifremer.fr

Marine bivalve molluscs represent an economically important class of organisms in which the lack of a cell line and stable system for gene transduction has hindered studies of molluscan molecular and cellular biology. To date, stable integration of foreign DNA has not been reported in oysters. Retroviral vectors containing the vesicular stomatitis virus envelope glycoprotein (pantropic vectors) can infect a wide range of different species. We tested whether pantropic vectors could mediate stable gene expression in primary cultured oyster cells. Primary cultured cells were initiated from hearts and from 18 h-old dissociated embryos of *Crassostrea gigas* and *Crassostrea virginica*. Cells were cultured in 2X L15 medium plus 10% fetal calf serum (750 mOsm) at room temperature. Cells were infected with pantropic vectors containing the luciferase reporter gene driven by different heterologous promoters and maintained for 3 to 31 days in culture until analysis by PCR and luciferase assay. Among the heterologous promoters tested, the highest levels of luciferase expression were obtained from the long terminal repeats of Moloney murine leukemia virus and Rous sarcoma virus. These experiments demonstrate that the pantropic retroviral vectors are a suitable tool for introduction and expression of foreign DNA in cultured oyster cells. Experiments are in progress to use these pantropic vectors to express oncogenes in oyster cells to create an immortalized oyster cell line.

I-1007

Medium Constituents Promoting the Attachment and Spreading of Cells of Eastern Oysters. J.F. LA PEYRE, Y. Li and J.T. Buchanan. Department of Veterinary Science, Louisiana State University, Baton Rouge, LA 70803, USA. Email:JLAPEYRE@AGCTR.LSU.EDU

Low cell adherence, cell detachment and eventual lysis of cells in suspension are common problems encountered in maintaining oyster primary cultures. Most normal cell lines are anchorage dependent meaning that they divide only in monolayer cultures attached to a substrate. It is therefore likely that the establishment of an oyster cell line will depend on the improvement of culture conditions promoting the attachment and spreading of oyster cells. We have recently shown that poly-D-Lysine significantly increased the attachment and spreading of oyster heart cells compared to untreated tissue culture plates (Falcon(r) and Corning(r)) after one day in culture. However, there was a general decrease in cell attachment after five days of culture which was attributed in part to the sub-optimal culture medium used (JL-ODRP-4). A new medium was developed by testing the effects of more than 30 ingredients individually and in combination on cells maintained in the optimized oyster basal medium LA-1. Our results indicate that a number of ingredients tested, such as bovine serum albumin, soybean trypsin inhibitor, catalase, vitamin E and mucin, significantly increased the attachment and spreading of oyster heart cells. We have now optimized a defined medium (LA-2) for oyster heart cells which dramatically improves cell attachment and spreading as compared to JL-ODRP-4 or a commercial medium commonly used to maintain oyster cells (L-15, Leibovitz).

I-1008

Development of a Culture Medium for Cells of the Eastern Oyster, *Crassostrea virginica*. J.F. LA PEYRE and Y. Li. Department of Veterinary Science, Louisiana State University, Baton Rouge, LA 70803, USA. E-mail:JLAPEYRE@AGCTR.LSU.EDU

The optimization of a culture medium for oyster cells may be a critical factor for the development of a cell line. We have recently optimized a medium (LA-2) by screening more than thirty chemically defined ingredients individually and in combination. This medium was superior to the commercial medium L-15 (Leibovitz) with and without fetal bovine serum (FBS). Addition of low concentrations of FBS (i.e., 2 and 4%) to LA-2 did not benefit oyster cells while the addition of higher concentrations of FBS (i.e., 6 and 8%) to LA-2 was detrimental. To further improve the medium LA-2, we have tested a number of undefined medium supplements (e.g., lactalbumin hydrolysate, peptone, yeastolate, egg yolk, oyster plasma) reported to be beneficial to oyster cells. Their effects on oyster heart cells maintained in LA-2 were first evaluated individually over a broad range of concentrations. Evaluation was done by comparing cellular metabolic activity (dehydrogenase) and by observing the morphology and contractility of the cultured cells. In a second set of experiments the combined effects of selected ingredients were determined using the statistical optimization approach based on a Plackett-Burmann statistical design, and an improved medium LA-3 was formulated. Finally, the benefit of serum from various sources (e.g., calf, fish, chicken, goat, porcine) to oyster cells in LA-3 is being evaluated to finalize the formulation of an optimal medium for oyster cells.

I-1009

In Vivo and In Vitro Effects of a Fat Body Extract on *Spodoptera littoralis*. G. SMAGGHE [1], M. LOEB [2], L. Tirry [1]. [1] Laboratory of Agrozoology, Ghent University, B-9000 Ghent, Belgium. [2] Insect Biocontrol Laboratory, USDA-ARS, Beltsville, MD 20705. E-mail: GUY.SMAGGHE@RUG.AC.BE.

A biological activity assay with a *Heliothis virescens* fat body extract (FBX) at 1% by oral feeding revealed 52% mortality of last-instar larvae of the cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). This polyphagous pest is of worldwide economic importance in cotton, vegetables and ornamentals, and resistant populations cause severe problems in various countries. With FBX at 10%, a reduction of pupation of 92% was scored as compared to controls. When lowering the concentration of FBX to 0.1 and 0.01%, toxicity was lost. To evaluate the action of FBX, we tested the effect with in vitro cultured imaginal wing discs that were incubated with different concentrations of 20-hydroxyecdysone (20E) (from 10 μM to 1 nM). Imaginal wing discs are cell masses that give rise to the adult wings during metamorphosis in holometabolous insects. In all cases, FBX at 25 μl/ml clearly promoted the development in imaginal wing discs. With 20E at 1 μM alone 58% of cultured discs showed evagination, while this percentage was 100% with the addition of FBX. About 50% of evagination was already scored in the case of 20E at 0.01 μM in combination with FBX. These first results indicate that FBX shows toxicity to Lepidoptera, and it promoted development in cultured imaginal wing discs in vitro. However, further research is needed to elucidate its mode of action and its possible use in the control of pest insects.

I-1010

Immunohistochemical Localization of MDF1 (Midgut Cell Differentiation Factor1) in Midgut Cells of *Heliothis virescens*. SHINTARO GOTO[1], Makio Takeda[1], Raziel. S. Hakim[2] and Marcia J. Loeb[3]. [1]Kobe Univ. Kobe, Hyogo, 6578501, Japan. [2]Dept. of anatomy, Howard Univ. Washington DC 20059 USA. [3]IBL, USDA, ARS, Beltsvile, MD 20705 USA. E-mail: mloeb@asrr.arsusda.gov

Insect midgut is not only the most important organ in the digestive system but also the entry point for Bacillus thuringiensis and other microorganisms. However, the physiology of the mechanisms that regulate midgut cell proliferation and differentiation is lacking. Methods for culturing lepidopteran midgut cells have been reported (Sadrud-Din, Hakim and Loeb, 1994), greatly stimulating this line of research. Midgut differentiation factor (MDF1) was purified from conditioned medium used for Manduca sexta midgut cell culture (Loeb et al., 1999). This polypeptide has homology to portions of bovine fetuin protein and stimulates cultured midgut stem cells of M. sexta or Heliothis virescens to differentiate.A polyclonal antiserum against MDF 1 was raised in a rabbit. MDF 1-like immunohistochemical reactivity was found in the cultured cells. Twenty four hours before molting from 4th instar to 5th instar (slipped head stage), a period when the stem cells in the larval midgut are undergoing rapid mitosis and differentiation in vivo, the number of cells stained by this serum increased. The only cell type that responded positively to the antibody was the mature columnar cell.

I-1011

Establishment of A Novel *Bombyx mori* Cell Line that Reacts Hemolympt and Changes Cell Form. S.IMANISHI[P], S.Tomita[1], M.Kiuchi[Q] and M.Kamimura[2]. [P]Department of Insect Genetics and Breeding,[Q] Department of Sericulture. National Institute of Sericultural and Entomological Science, Ohwashi, Tsukuba, Ibaraki, 305-8634 Japan. E-mail:imanishi@nises.affrc.go.jp

Many insect cultured cell lines have been reported since *Antheraea eucalypti* cell line. However, cultured cells are being analyzed as a tool of the research of susceptibility and proliferation of insect virus. Here we report embryonic cultured cell line of the Silkworm, *Bombyx mori* that was established from embryonic heads. This cell line named NISES-BoMo-BEN5 was derived from *gGominhaku* silkworm strain. After beginning of primary culture on the date of Mar 1997, this cell line was transferred to new flask firstly on May 1998. Now 10 times of subculture are counted. A period of subculture is 2 to 3 months in without medium exchange at 20°C. This cell line makes a network like oligodendroglia and astroglia. Spherical cells makes a network and then proliferate when cultured in the medium of EXCELL-400 that supplemented 10% of fetal bovine serum and 3% of heat-inactivated silkworm hemolymph, but cells cultured in the medium that supplemented 10% of hemolymph, change their forms immediately not to be uniform among these cells. Conditioned medium, of course, promoted to make their networks. BmPP (*Bombyx mori* Paralytic Peptide) stimulated a proliferation of this cultured cell. 10 micro mole of BmPP gave the almost same growth effect as 3% contents of hemolymph supplemented to EXCELL-400+3%FBS medium. There should be contained in some substances for cell differentiation and growth in hemolymph. This cell line, indeed, appears as an effective system for investigation of some substances in hemolymph.

I-1013

Juvenile Hormone and Juvenile Hormone Mimics Inhibit ^{14}C-GlcNAc Uptake and Proliferation in an Indian Meal Moth Cell Line Derived from Wing Imaginal Discs. H. OBERLANDER[a], C. E. Leach[a], and E. Shaaya[b]. [a]Center for Medical, Agricultural and Veterinary Entomology, Agricultural Research Service, United States Department of Agriculture, Gainesville, Florida 32604. [b]Agricultural Research Organization, Bet Dagan, Israel. Email: hoberlander@gainesville.usda.ufl.edu

Although mimics of juvenile hormone (JH) were the first Insect Growth Regulators evaluated for practical control of pest insects, in vitro studies with these agents have been very limited. Initial problems with stability and solubility were dealt with by combining juvenile hormone with a carrier protein for organ culture experiments. More recently, there has been some success in demonstrating direct effects of JH and JH mimics on established cell lines(Willis, In Vitro: Cell. Dev. Biol. 33, 86A, 1997; Iwabuchi, In Vitro: Cell. Dev. Biol. 35: 612, 1999). We found that JH and JH mimics inhibited ^{14}C-GlcNAc uptake and proliferation in the wing imaginal disc-derived cell line, IAL-PID2 (see also Oberlander et al., J. Insect Physiol. In Press). The most consistent response obtained in our studies was inhibition of cell proliferation, in the absence of 20- hydroxyecdysone. JH-I, JH-III, methoprene, fenoxycarb, and farnesol significantly inhibited cell proliferation after 3 days of exposure of the cell line to these agents. Linoleic acid had no effect on proliferation in these cultures. In addition, fenoxycarb inhibited ^{14}C-GlcNAc uptake by the cells even when cultured in the presence of 20-hydroxyecdysone or RH-5992(tebufenozide). Thus, the PID2 cell line demonstrates responsiveness to JH and its mimics through inhibition of various processes in the presence or absence of ecdysteroids.

P-1001

Optimised Metabolic Flux Distributions in *Catharanthus roseus* Cultures. X. HUANG, S. Cenkci and F. Mavituna. Chemical Engineering Department, UMIST, UK.E-mail:x.huang@stud.umist.ac.uk

Catharanthus roseus (Madagascar periwinkle) is the source of at least 200 pharmaceutical agents such as vincristine, vinblastine, ajmalicine, serpentine, catharantine and vindolene. Yields of these compounds when extracted from the whole plant are very low: 0.3–0.5% for ajmalicine and serpentine, 0.001% for catharanthine and vindolene, 0.0005% for vinblastine and vincristine, based on the plant dry weight. Metabolic engineering can therefore be a very effective approach to improve yields either in the plants or/in plant cell/tissue cultures via targetted genetic engineering or bioprocess engineering optimisation. For this purpose, we developed a computational model using a commercial optimisation software in order to obtain metabolic reaction fluxes for different physiological and process conditions and compared the results with our experimental data obtained from *C. roseus* cell cultures. We described the metabolism of *Catharanthus roseus* using more than 170 stoichiometrically balanced equations. These involved glycolysis, TCA cycle, pentose phosphate shunt, electron transport system, respiration, photosynthesis, the biosynthesis of amino acids, cofactors, nucleotides, fatty acids, phospholipids, cellulose, as well as the secondary metabolism leading to the production of ajmalicine, serpentine, catharanthine, vindolene, vincristine and vinblastine. The optimisation programme was then used to solve the pseudo-steady state metabolic flux balance equation. The solution gave the values of the metabolic fluxes (specific reaction rates) for 170 reactions including the substrate uptake and product excretion rates. The comparison of these with the experimental values indicated the biological potential as well as some interesting pinch points. These can be used for targeting genetic engineering manipulations as well as designing better media, strategic planning of precursor feeding and in vitro operating conditions for increased product formation.

P-1002

Production of Pilocarpine by *Pilocarpus pennatifolius* cultures. C.W. TANG and F. Mavituna. Chemical Engineering Department, UMIST, Manchester, UK. E-mail:chi.w.tang@stud.umist.ac.uk

Pilocarpus pennatifolius is a tree that grows wild in South America accumulating imidazole alkaloid pilocarpine in its leaves. This chemical is widely used in opthalmology in galucoma treatment. Since the chemical synthesis or biochemical routes to produce this compound have proved very difficult and uneconomical, it is obtained by extraction from the leaves. Plant tissue culture techniques can provide an alternative for the in vitro production. It is difficult however, to produce cell cultures from a woody plant species. We initiated callus from the leaves of *P. pennatifolius* using Schenk and Hildebrandt and Lloyd and McMown woody plant media supplemented with 2,4-D and NAA. Embryogenic and root cultures were also initiated from callus using different media. The suspension cultures normally consisted of very large aggregates. We could obtain fine cultures however, by using a homogenisation method. Suspension and immobilised cell cultures were studied in both shake flasks and a bioreactor. A production medium was designed to initiate pilocarpine production. A maximum of 124 mg/L of pilocarpine was detected in suspension cultures.

P-1003

Strategies for *in vitro* Taxol Production by *Taxus* spp. E. ZALAT, C. W. Tang and F. Mavituna. Chemical Engineering Department, UMIST, Manchester, UK. E-Mail: mjkpgzez@fs1.ce.umist.ac.uk

Our main strategy for economically viable *in vitro* taxol production involves the sequential stages of fast callus growth, fast suspension culture growth, *in situ* immobilisation of suspension and/or callus cultures in bioreactors, production media design, continuous product removal. We initiated callus cultures of *Taxus media* and *Taxus cuspidata* using different ex-plants, different growth regulators both in complete darkness and light, establishing several fast growing calli lines. The effect of media, solidifying agent, container, antioxidant and light on the callus growth rate were also studied. Kinetics of growth, substrate and oxygen uptake, in shake flasks and bioreactors were then studied. Suspension cultures were immobilised in the open pore network of reticulated polyurethane foam particles and sheets *in situ* in different bioreactor configurations. Our current studies include computational optimised metabolic flux analysis in order to determine the composition of production media, precursor feeding and operational strategies, as well as continuous removal of product from immobilised cell cultures in bioreactors run in repeated-batch mode (drain-and-fill).

P-1004

Bioflavonoid Production in Suspension Cultures Expedites Recovery of a Complex Series of Condensed Tannins. M. A. L. SMITH, F. E. Kandil, and D. S. Seigler. Departments of Natural Resources & Environmental Sciences, and Plant Biology, University of Illinois, Urbana, IL 61801. Email: imagemal@uiuc.edu

The flavonoid group of polyphenolic plant products, in particular the complex proanthocyanidins, have been linked to significant inhibition of heart disease, cancer, urinary tract infection, and age-related disorders. Despite heightened research attention and consumer interest in fruits, dietary supplements, and other products which contain health-protective proanthocyanidins, conclusive determination of bioactive flavonoid entities has not been accomplished, in part due to multiple inherent complications associated with isolation of proanthocyanidins from fruits. A continuous cell suspension of *Vaccinium pahalae*, once induced to express anthocyanins, served as a ready donor for a series of epicatechin monomers, dimers, trimers, tetramers and higher molecular weight condensed tannins. Following vacuum column chromatography of the cell culture extracts, the structures and the molecular weights were established by NMR (^{1}H and ^{13}C) and FAB MS. Cell cultures were free of interferences from other plant compounds such as enzymes or sugars (>75% of total weight of fruit extract). Epicatechins from cell culture were characterized by 4,6 connections, rather than the more common 4,8 connections. Isolated, identified tannin compounds provided useful standards for related extraction and fractionation work with in vivo plant materials, and provided significant inhibition in a series of bioactivity assays.

P-1005

Evaluation of Different Methods for Cryopreservation of Taxus media Cell Suspension and Its Secondary Metabolite Profile. T. LIAO, N. Stoynov, J.P. Kutney, E.A. Polishchuk. Department of Chemistry, University of British Columbia, Vancouver, B.C.,Canada, V6T 1Z1. Email: elena@chem.ubc.ca

Renewed interest in Taxol(r) as an important anticancer agent led us to extensive work on its potential production in Taxus media cell suspension culture. Chemical stability of the culture depends on multiple factors of incubation and requires tedious and constant maintenance. We used two different approaches for cryopreservation of Taxus cell suspension in order to preserve not only the culture itself, but also its secondary metabolite profile. The first approach was to encapsulate the cells in calcium alginate followed by culturing them in sucrose enriched medium and then air dehydrating the alginate coated cells over silica gel before immersion into liquid nitrogen. We were not able to obtain viable plant culture because this method consists of many steps with high risk of contamination. The cell suspension culture was successfully recovered after preservation via a simpler, alternative method. It involved preculturing the cells in 6% sorbitol or mannitol, then adding cryoprotectants (glycerol or dimethyl sulfoxide), followed by slow cooling to −180°C. The resulting plant suspension was compared with the original culture for viability with fluorescein diacetate. The effect of various cryoprotectants used for culture recovery has been discussed. We monitored eight different taxane standards by HPLC analysis and compared their retention times with the retention times of the signals from both the revived and control cell suspensions. Four solvent systems were employed and comparison of the HPLC profiles for both cultures has shown similar peaks; however, the relative concentrations after recovery were different.

P-1006

Bulb Growth and Stem Foundation in Lily Bulbets Regenerated *In Vitro*. [1,2] M.M. LANGENS-GERRITS, [3]A.F. Croes, and [2] G.J. de Klerk. [1] Bulb Research Centre/ [2]Centre for Plant Tissue Culture Research, PO Box 85, 2160 AB, Lisse, THE NETHERLANDS; [3]University of Nijmegen, THE NETHERLANDS. E-mail: merel.langens.lbo.agro.nl

In tissue culture, plantlets should be produced that perform well after transfer to soil. We study whether micropropagated lily bulbets can be 'programmed' for fast growth after planting. We have identified two main factors that determine performance after planting. The first factor is size at the time of planting: large bulbets grew faster than small bulbets. Large bulbets could be obtained by culture in the dark, but high sucrose concentration and in particular, by using large initial explants. Uptake experiments with ^{14}C-sucrose showed that in large explants about 30% of the weight increase (mg DW) of the regenerating bulbets was accounted for by uptake of sucrose from the medium whereas in small explants this figure was 60–70%. Apparently, ample presence of carbohydrates or other compounds in the explant support growth of the bulbets. In pulse-chase experiments, we found that sucrose that had not been transported immediately to the regenerating bulbets, remained in the explant or was respired. The second factor enhancing growth after planting is the presence of a stem instead of leaf-blade bearing scales. We studied which factors during tissue culture enhanced stem formation. Culture for a long period (8–12 w) at high temperature (25°), followed by a short period (2–4 w) at moderate temperature (15°) was essential. Large bulbets more often formed a stem than small bulbets. Stem formation was enhanced by high sucrose concentration and by regeneration from a large explant. Murashige and Skoog-salts inhibited stem formation. For all three factors we observed that increased stem formation coincided with low endogenous levels of P and S in the regenerating bulbets.

P-1007

Direct Shoot Regeneration and Microtuberization in Wild Cyclamen persicum Mill. Using Seedling Tissue. M. AL-MAJATHOUB and N.S. Karam. Faculty of Agriculture, Jordan University of Science and Technology, P.O. Box 3030, Irbid, Jordan. E-mail: karam@just.edu.jo

The influence of cutting method of leaves on direct regeneration in wild cyclamen was studied. Wounded blades, blades with midrib, blades without midrib, central lamina, and petiolated lamina of *in vitro* leaves were cultured on solid MS media containing 0.1 mg l^{-1} NAA and 0.22 mg l^{-1} TDZ. There was a significant effect of leaf cutting method on shoot regeneration; shoots regenerated on all explants except those consisting of blades without midrib. The greatest regeneration percentage (88%) was obtained on leaves with midrib. The influence of type of explant and concentrations of BA and sucrose on microtuberization was also studied. *In vitro* seedling tuber, petiole, cotyledon, and root explants were cultured on solid MS media supplemented with 0.1 mg l^{-1} NAA and different concentrations of BA. No tuberization occurred on tuber or petiole explants. Root explants cultured on media containing 1 mg l^{-1} BA exhibited the greatest microtuberization response. Mirotubers were able to sprout within six weeks following initiation of cultures. *In vitro* microshoots and seedling root explants were cultured in liquid and solid MS media, respectively, containing different concentrations of sucrose. Microshoots failed to produce tubers, whereas root explants showed the capacity for tuber formation. Sucrose concentration had a significant effect on tuberization; 3% sucrose induced 100% tuberization, whereas 9 or 12% sucrose suppressed tuberization. Average weight and size of tubers peaked at 6 and 3% sucrose, respectively.

P-1008

Selection of plants for phytoremediation. A. GERTH and A. Böhler. BioPlanta GmbH Benndorfer Landstraße 2, 04509 Delitzsch, GERMANY. E-mail: BioPlanta@t-online.de

The activities of plants and plant/microbe associations may offer a viable means of accomplishing remediation of wastewater and the in-situ remediation of contaminated soils. Two uses of plants for phytoremediation are reported here. The following target toxicants were examined: heavy metals in the helophyten rhizospheres, the degradation of phenolic compounds and persistent organic pollutants in plant-microoganism systems, degradation of TNT in soil through combined bio- and phytoremediation. In one set of studies, the ability of plants for uptake heavy metals, radionuclides and phenolic compounds from contaminated wastewater are investigated. The ability of plants to absorb and metabolize pollutants was examine in laboratory and scale up to pilot-scale and full-scale treatment. The BioPlanta GmbH carried out an extensive initial test to determine the tolerance limits, degradation and adsorption rates of organic and anorganic contaminants by helophyten under derfined biotic and abiotic conditions (in vitro). Examinations with plants for decontamination of heavy metal contaminated soil and sludge have shown less accumulate rates because of the plant avaibility of heavy metals. Plants have shown the capacity to with-stand relatively high concentrations of organic chemicals without toxic effects, and they can uptake and convert chemicals quickly to less toxic metabolites in some cases. The use of the association between plant and microoganism for in situ bioremediation of TNT in soil was part of a second research programm in technical and pilot-scale. The investigations belongs to the use of white rot fungi as a soil inoculum. Recent studies indicate that TNT is accelareted in the root zone. Actively growing plants increase soil organic carbon, bacteria, and m

P-1009

Growth and Yield of Clonally Propagated Papayas Compared to Multiple-Seedling Transplants. M. FITCH[1], T. Leong[2], L. Akashi[2], S. Ferreira[3], and P. Moore[1]. [1]Pacific Basin Agriculture Research Center. ARS, USDA, 99–193 Aiea Hts. Dr., Aiea, HI 96701, [2]Hawaii Agriculture Research Center. 99–193 Aiea Hts. Dr., Aiea, HI 96701, [3]University of Hawaii, Department of Plant Pathology. 3190 Maile Way, Honolulu, HI 96822. Email: MFITCH@HARC-HSPA.COM

Hybrid papaya trees (*Carica papaya*) segregate 1 female to 1 hermaphrodite. Producers in Hawaii prefer the hermaphrodite fruit for its distinctive pyriform shape and better handling due to its seed-filled fruit cavity. To ensure fruit production by hermaphrodite trees, producers normally grow several plants per hole for 4 to 5 months, until the plants flower and can be sexed before thinning to a single hermaphrodite. This method of orchard establishment is wasteful of labor, water, and fertilizer and plants appear to be affected adversely by crowding and competition. The validity of this observation is supported by data from Australia and South Africa showing that clonally propagated papaya plants produce fruit sooner and lower on the fruit column. We wished to quantify, under Hawaii conditions, differences in growth and yield of clonally propagated plants compared to multiple-planted seedling papayas. All studies were based on the papaya hybrid cultivar Rainbow, a line engineered for resistance to papaya ringspot virus. Clonal propagation was through cuttings and micropropagation in tissue culture. Sexing of young seedlings for use as transplants was accomplished by PCR amplification of leaf DNA extracts to visualize the sex-determining locus. Trials were conducted at three sites with comparisons of either three or four treatments. The three treatments at all sites were multiple-planted seedlings, micropropagules, and cuttings. The fourth treatment included at two of the sites was the PCR-sexed transplanted seedlings. Plant growth and fruit yield of all treatments varied among the sites for all treatments. However, at all sites clonally propagated plants fruited 2 to 3 months earlier and lower on the stem than did the multiple-planted seedlings. The single transplants were intermediate between the groups in development. Although the multiple-planted seedlings were slower to initiate flowers, yields converged so that within 2 to 3 months of bearing or at 13 to 14 months of age, the multiple-planted seedlings yielded the same as the clonally propagated plants. Nevertheless, the clonally propagated plants remained shorter in stature, thus easier to harvest as the orchards matured. After 1 year of harvests, total yield of the clonally propagated plants was greater than that of multiple-planted seedlings. At present, a shortage of Rainbow seed prevents orchard establishment and a new awareness of the benefits of early and prolonged harvest of clonally propagated plants has encouraged the start up of small businesses to supply clonal plants. An analysis of the costs and returns to farmers of the various methods for establishing papaya orchards is under way.

P-1010

Vegetative and fruiting Comparison of Tissue culture-Derived and Conventionally Propagated Date Palm (Phoenix Dactylifera L.)cv. Barhi Trees. ABDELRAHMAN S. AL-WASEL, Dept. of Hort. and Forestry, Colleg of Agr., King Saud University, P.O.Box, 1482, Al-Qassim, Sudi Arabia.

The study was conducted to assess the uniformity of tissue culture-derived trees to the conventionally propagated trees of date palm cv Barhi. Tissue cultured plants were more vigorous, uniform and produced significantly much more primary, secondary and aerial off-shoots in comparison to off-shoot produced plants. The other vegetative characteristics, such as leaf length, leaflet zone, leaflet length, leaflet angle, thorn area, thorn length, thorn angle, were not reliable traits to assess the uniformity of tissue cultured plants to conventionally propagated plants since there were significant differences not only between the tree type (either tissue culture derived or conventionally propagated plants)but also among the trees of the same tree type. In trems of fruiting, number of trees transplanted in 1992 and later failed to set fruits and the flowers were abnormal with more than 3 carpels (sometimes reached up to 7, normal flower has 3 carpels). Therefore, further study is needed to confirm whether tissue culture technique produces identical propagules of elite date palm cultivars or would result in somatic variation among in vitro produced plants.

P-1011

Micropropagation of the Threatened Medicinal Plant Allium wallichii (Kunth) in Nepal. P. MALLA and S. Malla. Institute of Pharmacognosy, University of Vienna, Centre of Pharmacy, Althanstrasse 14, A-1090 Vienna, AUSTRIA and Amrit Campus Tribhuvan University Kathmandu, NEPAL. Email: Prakash_malla@hotmail.com

Allium wallichii (Liliaceae), a perennial herb commonly known as Banlasun, has both medicinal and ornamental uses. It grows widely in the east central and western Himalayas of Nepal (1). Its roots and leafy portion are widely used in folk medicine against rheumatism, antibacterial and antifungal properties. Due to indiscriminate collection and over-exploitation of the natural resources, Allium wallichii is rapidly disappearing and is therefore listed under the endangered species of Nepal(2). In view of this, there is an urgent need for the conservation of this valuable species, and the use of micropropagation techniques might be a promising approach. In vitro-cultures of Allium wallichii were initiated from seed inoculated on half MS medium (3). These regenerated shoots were used as secondary explants for the establishment of a multiplication protocol. Longitudinal sections (LS) of shoots, whole shoots, and leaves were taken from six week old aseptically grown plantlets and cultured on MS medium supplemented with factorial combinations of various phytohormones (BAP, zeatin, and kinetin combined with IAA, NAA, and IBA). Among the different treatments, the best morphogenetic response was achieved on medium with 20-μM zeatin alone. For rooting, the in vitro-proliferated shoots were transferred to medium containing 10 μM IBA. Subsequently rooted plantlets could be successfully established under greenhouse conditions. Currently, field trials in Nepal with micropropagated Allium wallichii are in progress. Our results indicate that plant tissue culture can be successfully applied to this endangered species. Micropropagation with subsequent field culture may be an alternative to the collection from natural sites and may allow the production of large amounts of homogenous crude drug material for medicinal use.

P-1012

Conservation of virus-free seed potato of four cultivars in three Northeast locations in México. H. GUTIERREZ M., C.G.S. Valdés L. and L.A. Moreno A. Fac. de Agronomía, UANL. Apdo. Postal 358, San Nicolás de Los Garza, N.L. México.

Free-virus certified seed potato production requires time and a proper crop management. Northeast Mexico presents variation in altitude and this would make possible to have two potato crops per year for seed purposes, one in summer (s) at high land locations and other in autumn-winter (a-w) at low land locations; on the other hand, potato crop is located in spoted areas, but it may be planted in locations at wich this crop has not been planted or it has been planted some in a while where it is expected to have a low inoculum of potato viruses in environment. This work was carried out to know if this conditions would be used to speed certified free-virus seed potato in one location planted in s at 2000 m above the sea level (asl), La Asencion, N.L., and two locations planted in a-w, Marín N.L. and Sabinas N.L., at 45 m (asl). ELISA analyses were made for PVX, PVY, PVA, PVS, PVM and PLRV in microtubers and minitubers produced from in vitro plantlets of Atlantic, Mondial, Carola, and Herta cultivars, as well as, in Basic (pre-Elite) at La Asencion, and in the Registered (Elite I) and Registered I (Elite II) categories of seed potato produced at Marin N.L. and Sabinas N.L. For all cultivars, seed potato harvested in field at all locations was virus-free like in vitro plantlets, microtubers and minitubers from it was developed. These results indicate that the proposed approach to speed free-virus potato seed productions in Northeast Mexico would be validated under commercial plantings.

P-1013

PLANT GENETIC INSTABILITY DETECTED BY MICROSATELLITES-PRIMERS X. J. LEROY[1], K. Leon[2] and M. Branchard[1]. [1] Biotechnology and Plant Molecular Biology Unit, ISAMOR, 29280 Plouzané, France [2] Rhobio, Génoplante, 91000 Evry, France. E-mail: xavier.leroy@univ-brest.fr

The process of somaclonal variation is believed to require multiple genetic and/or epigenetic events which affect patterns of expression, or result in mutational alteration of genes. Various molecular mechanisms may be responsible for the DNA mutation and genetic instability leading to the development of variations. Such molecular mechanisms would include DNA damage and mutation, alteration of a cell's ability to repair damaged or mutated DNA, alteration of genes for cell-cycle control mechanisms, DNA methylation. However, few mechanisms of somaclonal variation have been described. While method for detecting microsatellite instability is an important tool in genetic characterisation of cultivars, such method do not detect major forms of genomic instability characterised by molecular aneuploidy arising from deletions, amplifications, translocations, insertions, recombination, and chemical alteration. The present article provides a method for detecting genomic instability. ISSR technique might be useful in a method for detecting a major type of somaclonal variation. For the method of detecting chromosomal breaks in genomic sequences located between microsatellites, $(CAA)_5$, $(CAG)_5$, $(GACA)_4$ and $(GATA)_4$ primers were synthesised. DNA was prepared from *in vitro* culture samples. Alterations (changes in banding patterns) may be visualised. Calli alterations were detected as gains, losses in the pattern of amplified bands. The number of genomic lesions might be expected to be greater in calli of a higher stage if such lesions occurred stochastically during cellular proliferation. These data suggest that initiations of genomic instability can occur at an early stage of calli formation. One clone was homologous to an *Arabidopsis thaliana* cDNA and a human cellular proliferation regulator gene. Thus cloning and sequencing of the altered bands can be used for the identification and the mapping of altered regions/genes of the genome. Precise definition of the kind of mutations detected may further yield important clues to novel somaclonal phenotypes.

P-1014

Induction of a Filamentous Form of *Agrobacterium*. K.R. Finer, K.M. Larkin, B.J. Martin and J.J. FINER. Department of Horticulture and Crop Science, The Ohio State University, Wooster, OH 44691. E-mail: FINER.1@OSU.EDU

The use of *Agrobacterium* for plant cell transformation has increased over the past few years as a result of our greater understanding of the T-DNA transfer process, its ease of use compared to other transformation methods, and the desire to obtain more precisely controlled DNA integrations. Through the development of strains that constitutively express certain *vir* genes, *Agrobacterium* can now be routinely used for introduction of DNA into cells and tissues of many plants, even though some of these plants are not considered to be natural hosts for this plant pathogen. Although the host-range limitations of *Agrobacterium* have been largely reduced, very little is still known about the physical relationship between the bacterium and the targeted host cell. In order to gain a better understanding of the initial events resulting from contact of the bacterium with plant tissue, we attempted to observe structural changes, which occur during co-cultivation of *Agrobacterium* with plant tissues. Following co-culture of *Agrobacterium* with a variety of different plant tissues, changes in both colony and bacterial structural morphology were observed. Bacterial colonies, grown in the presence of plant tissue, became opaque and appeared to grow as a thick mat of cells. A single bacterial colony would often grow to fill an entire 100 mm diameter Petri dish. Ultrastructural observations of the bacteria in these colonies revealed the formation of a predominantly filamentous form of the bacterium. The bacteria ranged from 5 to 100 microns long as compared to 2 microns in the non-filamentous form. The filamentous form was observed 2–3 days after co-culture and only if the bacteria were either in direct contact with or in close proximity to living plant tissues. The filamentous form was observed with both wild type and engineered *Agrobacterium* strains. We believe that the conversion to the filamentous form may be associated with the infection process and permit the bacterium to span large distances when looking for a suitable entry site.

P-1015

Use of fluorescence *in situ* hybridization for mapping of transgenes and screening of homozygous plants in transgenic barley (*Hordeum vulgare* L.). H.W. CHOI, P.G. Lemaux and M.-J. Cho. Dept. of Plant and Microbial Biology, Univ. of California, Berkeley, CA 94720, USA. E-mail: choihw7@uclink4.berkeley.edu

Introduced *uidA and gfp* genes were localized using fluorescence *in situ* hybridization (FISH) on the metaphase chromosomes of transgenic barley produced by microparticle bombardment of immature embryos. Of the 13 independent transgenic lines, 7 were *uidA*-containing and 6 contained *gfp* (3 diploid and 10 tetraploid lines). All lines had 3 or more copies of the transgenes and 12 lines had different integration sites; at a gross level it appeared that no preferential integration patterns of foreign DNA were present. Only one integration site was detected on a single chromosome in diploid ($2n=2x=14$) T_0 plants of events with a 3:1 segregation ratio; homozygous plants obtained in T_1 or later generations of these events had 2 signals on a pair of homologous chromosomes. We previously reported a high frequency of ploidy level change in transgenic barley plants. Current FISH analyses showed that tetraploid ($2n=2x=28$) T_0 plants with a 3:1 segregation ratio also had one integration site on a single chromosome; this indicates that cells were already tetraploid at the time of DNA integration. In contrast, one tetraploid T_0 plant with a 35:1 segregation ratio in the T_1 generation had 2 signals on a pair of homologous chromosomes, indicating that cells were diploid and became tetraploid after DNA integration. One tetraploid T_0 line with a segregation ratio consistent with being a homozygote (45:0) had 2 signals at 2 loci on separate chromosomes. we conclude that the application of FISH technique for analysis of transgenic plants is useful to perform gross localization of transgene(s) and screen for homozygous plants.

P-1016

Development of Efficient Transformation Systems for Monocotyledonous Crop Species Using Highly Regenerative, Green Tissues. M.-J. CHO. Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA. E-mail: mjcho@nature.berkeley.edu

Highly regenerative, green tissues were generated and maintained on MS-based media containing 2,4-D, BAP and high level of cupric sulfate under dim light conditions. Using highly regenerative tissues as transformation targets, we generated a large number of independently transformed lines for several monocot crop species that gave rise to transgenic plants. For oat transformation, 84 independent transgenic events were obtained from 327 individual explants, giving an transformation frequency (TF = # independent lines/total # explants) of 26%; all events (100%) were regenerable. TFs of spring barley cultivars, Golden Promise, Galena and Harrington, were 0.9 to 1.7. The use of these transformation systems also resulted in successful transformation of a spring wheat cultivar, Bobwhite, with 5.5% effective transformation frequency (ETF = # fertile, regenerable independent lines/total # explants). For the two previously recalcitrant spring wheat cultivars, Anza and Yecora Rojo, TFs were 4.9% and 4.6%; for a recalcitrant winter cultivar, Karl, the TF was 3.8%. The TFs for maize cultivars, H99 and B73, a recalcitrant inbred line, were 7.1 and 2.6%, respectively. Nine independent transgenic rice lines were obtained from 133 pieces of tissue, giving a 6.8% TF. The TFs for forage/turf grasses varied amongst the crop species; 7.5%, orchardgrass; 6.8%, tall fescue; 4.5%, red fescue; 5.1%, creeping bentgrass; and 2.0%, Kentucky bluegrass. Regenerability of the transgenic forage/turf grasses was 60 to 100%.

P-1017

Improvement of MAT (Multi-Auto-Transformation) Vector System for Generating Marker-Free Transgenic Plants. H. EBINUMA, K. Sugita, E. Matsunaga S. Endo, K. Yamada and A. Kawaoka. Central Research Laboratory, Nippon Paper Industries CO., LTD., 5-21-1, Oji, Kita-ku, Tokyo 114-0002, Japan. Email: LDW06374@nifty.ne.jp, 0880080@fr.nipponpaper.co.jp

In current systems, selective agents and the corresponding resistance genes are used for the selection of transgenic cells. However, these systems have three problems: (1) The selective agents have negative effects on proliferation and differentiation of plant cells. (2) There is uncertainty regarding the environmental impact of many selectable marker genes. (3) It is difficult to repeat transformation using the same selectable marker in order to pyramid desirable genes. The MATVS is designed to overcome these difficulties. The MATVS is based on a novel concept that oncogenes of Agrobacterium is used as a selectable marker to regenerate transgenic cells and select marker-free transgenic plants. Commonly oncogenes have not been used for the selectable marker gene to transform plants because the resulting transgenic plants exhibit a serious abnormal phenotype. The MATVS is designed to remove the oncogenes from transgenic plants after a transformation. In the MATVS, the chimeric ipt gene or the rol genes are combined with the site-specific recombination R/RS system to remove them from the transgenic cells after transformation. The R/RS system cassette is used for selection and called the hit and run cassette of the MAT vector. We report here improvements of the hit and run cassette. (1) second markers (NPTII, GUS, GFP) is inserted into a hit & run cassette to assist selection of transgenic plants. (2) an intron sequence of eucalyptus histone genes is inserted into the R genes to suppress dropouts of a MAT cassette during cultivation of Agrobacterium. (3) the ipt gene is fused with several different promoters to optimize the cytokinin level for the regeneration of transgenic cells. (4) the ipt genes are combined with iaaM/H genes to manipulate auxin/cytokinin ratio.

P-1018

A novel MADS-box gene is required for shoot bud induction in excised leaf cultures of *Paulownia kawakamii*. P.P. KUMAR and A.P. Prakash. Plant Morphogenesis Laboratory, Department of Biological Sciences, The National University of Singapore, 10 Science Drive 4, Singapore 117543. E-mail: dbskumar@nus.edu.sg

We describe the isolation and characterization of a MADS-box cDNA (*PkMADS*) from *Paulownia kawakamii* leaf explants undergoing adventitious shoot regeneration. Sequence analysis of the MADS domain of PkMADS protein shows high homology (80–90%) to StMADS11 and StMADS16 that are expressed in the vegetative tissues of *Solanum tuberosum*. mRNA gel blot analysis confirmed the expression of *PkMADS* in the shoot-forming cultures but no signal was observed in the callus-forming cultures. Transcripts of *PkMADS* were also detected in shoot apices but not in root apices and day 0 explants. Overexpression of *PkMADS* in *Paulownia* resulted in significant changes in phenotype, such as, leaf curling, reduction in leaf area and axillary shoot formation. In the antisense transformants, shoots were stunted, had altered venation and phyllotaxy and in some lines the shoot apical meristem appears to have been used up early during shoot development. Further, leaf explants from the antisense transgenic plants showed tenfold decrease in shoot regeneration than the explants from the sense transformants. The expression of *PkMADS* was restricted to the developing shoot primordia, and absent in the callus-forming tissues as revealed by in situ hybridization. Our results indicate that *PkMADS* gene is required for adventitious shoot formation in *Paulownia kawakamii*.

P-1019

Visualizing Green Fluorescent Protein. D.J. GRAY, N.J. Barnett, M.M. Van Aman, D.P. Weaver, K.T. Kelley, S. Jayasankar and Z.J. Li. Central Florida Research and Education Center, IFAS, Univ. of Florida, 5336 University Ave., Leesburg, FL 34748. E-mail: DJG@GNV.IFAS.UFL.EDU

&#65279peen Fluorescent Protein (GFP) increasingly is being used as a versatile marker to identify transgenic cells and to elucidate aspects of their function. The gene that encodes GFP was isolated from a jellyfish, *Aequorea victoria*, and has been found to be functional when introduced into a wide range of organisms. GFP contains a chromophore that is excited by exposing it to blue light; this causes the release of green fluorescence. Since green and blue wavelengths are co-mingled, visualization of green fluorescence is accomplished by filtering-out the blue light. A compound microscope equipped for epi- fluorescence utilizes transmitted light to view GFP in cells and tissues at relatively high magnifications. However, only recently have stereomicroscopes been developed that use reflected light to visualize GFP at the low magnifcations needed for screening putative transgenics. Such stereomicroscopes remain prohibitively expensive to justify for many applications. An alternative method to visualize GFP stereomicroscopically and macroscopically will be described.

P-1020

Characterization of differentially expressed extracellular proteins after *in vitro* selection for anthracnose resistance in grapevine. S. JAYASANKAR Z.J.Li and D.J. Gray MREC University of Florida 2725 Binion Road APOPKA FL 32703. E-mail: js@gnv.ifas.ufl.edu

Proembryogenic masses (PEMs) of grapevine (*Vitis vinifera* L.) 'Chardonnay' were recurrently selected with culture filtrate produced by *Elsinoe ampelina,* a phytopathogenic fungus that causes grapevine anthracnose. Analysis of spent culture medium for extracellular proteins revealed three new proteins to be differentially expressed by *in vitro* selected PEMs. Since the plants regenerated from *in vitro* selected cultures exhibited high degree of resistance to anthracnose fungus, a possible role for these proteins in anthracnose resistance is suggested. Previously *[In Vitro Cell. Dev. Biol.—Plant, 35(3) II, P-1031]* we described these proteins in embryogenic cultures. In the present study we further characterize the proteins and discuss their potential roles in regenerated plants.

P-1021

A study on the expression of the Grapevine *Vitivirus A* movement protein gene in transgenic grapevines. L. MARTINELLI[1], E. Candioli[1&2], F. Dalla Vecchia[2], A. Minafra[3], N. Rascio[2]. [1]Istituto Agrario, 38010 San Michele a/Adige (TN), Italy; [2]Dipartimento di Biologia, Università di Padova; [3]Dipartimento di Protezione delle Piante, Univ. & Centro Studio CNR Virus e Virosi delle Colture Mediterranee, Bary, Italy. E-mail: Lucia.Martinelli@ismaa.it

The movement protein gene of the grapevine *Vitivirus A* (GVA), both in sense or antisense orientations, has been inserted in *Nicotiana* and somatic embryos of *Vitis rupestris* (Martinelli and Mandolino, TAG 88: 621–628, 1994) and *Vitis vinifera* cv. Superior seedless (kindly provided by dr. A. Perl, The Volcani Center, Israel) after co-culture with *Agrobacterium tumefaciens* LBA 4404. Plantlets were regenerated, and molecular assays proved the exogenous gene insertion. In tobacco, R1 seedlings showed an interesting degree of protection from GVA infection in greenhouse tests (Martinelli *et al.*, Acta Hort., in press). In transgenic grapevines, assayes have been designed aiming to test the behaviour of the exogenous viral gene in the plant tissues, and molecular and ultrustructural analyses are carrying out. Our attempt to understand the mechanisms leading to pathogene derived resistance, as well as to gain knowledge of the events occurring in transgenic plants in the view of risk assessment, will be discussed. Authors wish to thank Prof. G. Martelli, dr. M. Komjanc, dr. D. Costa and dr. S. Festi for precious suggestions, and p.e. V. Poletti for technical support.

P-1022

Engineering Disease Resistance in Wheat by Cloning Defense Genes. AJITH ANAND[1], Tian Zhou[1], Regina D. Wamsley[2], Vasanth Janakiraman[2], Sujatha Prakash[2], Wanlong Li[2], Wenpin Chen[2], Natarajan Sakthivel[1], Bikram Gill[2], George Liang[3], Jyoti Shah[4], Harold N. Trick[2] and Subbaratnam Muthukrishnan[1]. [1]Department of Biochemistry, [2]Plant Pathology, [3]Agronomy, [4]Biology, Kansas State University, Manhattan, KS 66506. E-mail: ajith@ksu.edu

Pathogenesis-related (PR) proteins are an important part of the strategies evolved in plants to defend themselves against pathogen attacks and insect infestations. We are presently investigating the possibility of engineering wheat plants for the constitutive expression of gene(s) involved in these defenses. Genes/cDNA for defense proteins include several PR-proteins isolated from *Rhizoctonia*-infected rice plants and scab- infected wheat as well as the *NPR1* gene from *Arabidopsis thaliana*. We are employing the biolistic approach to introduce these genes in wheat embryogenic calli and screen the putative transgenic plants for the presence of transgene by PCR and for expression by western blot analysis with appropriate antisera and enzyme assays. Our earlier investigations indicated that plants engineered with a rice-thaumatin like protein showed a delayed progression of scab infection. Presently work is underway to introduce combinations of chitinases, $-1,3-glucanases, and TLP to identify the best combination(s) of PR proteins for enhanced resistance to fungal diseases (scab, leaf rust, Take-all) and also to analyze the effect of *NPR1* expression on resistance to different pathogens.

P-1023

Effect of Expression *hrpN* in Apple on Resistance to *Erwinia amylovora*. E. BOREJSZA-WYSOCKA, A.A. Kader, J.L. Norelli, D.W. Bauer, E.R. Garr, S.V. Beer and H.S. Aldwinckle. Depts. of Plant Pathology, Cornell University, Geneva and Ithaca NY 14456 and 14853. E-mail: eb31@cornell.edu

Fire blight caused by *Erwinia amylovora*, is the most serious bacterial disease of apple worldwide, and affects all plant parts. Harpin, encoded by the *E.amylovora* gene, *hrpN*, induces resistance when topically applied to apple tissues. The horticulturally desirable but fire blight susceptible, apple rootstock M.26 was transformed with 4 *hrpN* gene constructs to evaluate their potential for increasing resistance to fire blight. Regenerants were obtained on medium containing kanamycin (100 mg/L) and transformation was confirmed by NPTII ELISA and PCR analysis for presence of the *hrpN* gene. Transgenic lines with Pnos-*hrpN* (14 lines), Pnos-anti*hrpN* (14), Pnos-SS/*hrpN* (21), and Pgst1-SS/*hrpN* (28) were obtained. SS is the signal peptide sequence of the PR1 protein of tobacco. Eight of nine Pnos-*hrpN* lines were positive in western analysis for presence of harpin. Vigorously growing shoots of Pnos-*hrpN* transgenic lines of M.26 were inoculated with *E. amylovora* to evaluate their fire blight resistance. M.26 plants expressing harpin without SS were not significantly more resistant to fire blight than parent M.26 and there was no detectable association between the level of harpin expression and the level of disease development. This result was not unexpected since research with *Arabidopsis* has shown that induction of disease resistance by harpin appears to be enhanced by the presence of SS for transport of harpin to the intercellular space. M.26 lines transgenic for SS/*hrpN*, driven by Pnos and Pgst1, are currently being propagated for disease evaluation, as well as analysis of harpin expression and SAR induction.

P-1024

Caspase Inhibitors Block Pathogen Proliferation And Symptoms Of Compatible And Incompatible Plant-Pathogen Interactions. CRAIG RICHAEL, James E. Lincoln, Richard Bostock, David Gilchrist. Center for Engineering Plants for Resistance against Pathogens, University of California at Davis, Davis, CA 95616. Email: dggilchrist@ucdavis.edu

The central role of caspases in programmed cell death (PCD) was initially determined using potent peptide and macromolecular inhibitors. Though morphological and biochemical markers of programmed cell death are present in plants, the existence of caspases in plants remains unresolved. In addition to expression of apoptosis-suppressing macromolecular inhibitors like p35, we have used peptide inhibitors to elucidate the role of native plant caspases in plant-pathogen interactions. A number of tetrapeptide inhibitors used in our experiments (e. g. Ac-DEVD-CHO) specifically bind and diminish the proteolytic ability of animal caspases. Several inhibitors block non-host specific and host specific HR cell death elicited by avirulent bacteria without compromising resistance in plants. In compatible interactions the tetrapeptide inhibitors blocked the symptoms of common bacterial diseases of tobacco, bean and tomato. The inability of these bacterial pathogens to cause disease in the presence of the peptide caspase inhibitors was apparent from the lack of growth of the causal organism as determined by growth curves. The tetrapeptide inhibitors are not lethal to bacteria in vivo or when grown in artificial media in vitro. The broad-spectrum protease inhibitor leupeptin (Ac-LLR-CHO) and Ac-DEVD-COOH had no affect on the development of HR or compatible cell death. These results suggest that caspases exist in plants and that the molecular basis of PCD is conserved across kingdoms. The mechanism of cell death during hypersensitive cell death may be the same as that invoked during cell death leading to disease symptoms. The elicitation of PCD is important to necrotrophic pathogens to become pathogenic, however, HR cell death does not appear to be vital to resistance.

P-1025

Transformation and Characterization of a Thaumatin Like Antisense Gene of Barley in Wheat for Increased Fungal Resistance. A.PELLEGRINESCHI, M.M. Salgado, S. McLean, D. Hoisington, G. Fincher, S. Donner, and R. Osmond. Applied Biotechnology Center, CIMMYT, Ap.do Postal 6-641, 06600 Mexico.

Transformation of wheat (*Triticum aestivum*) via particle bombardment with an anti-sense Thaumatin-like gene derived from barley (*Hordeum aestivum*) resulted in a modified expression of the endogenous wheat thaumatin, in the transformed plants. Transgenic wheat plants were regenerated by co-transformation of immature embryos followed by selection on PPT. Stable integration of the genes in the genome, and inheritance in the progeny, were determinated by phenotypical analyses challenging the plants against a wide range of pathogens, PCR, and Southern-blot analysis. We recover over 63 independent events with 25–30% of co-transformation efficiency. The quantity and anti-fungal activity of the endogenous thaumatin like proteins were analyzed in T1 and T2 progeny plants. Western blot analyses showed different patterns of the wheat endogenous thaumatin-like proteins. Preliminary results indicated that some patterns increased resistance of transgenic wheat plants to Alternaria tritici. This relationship is being further investigated.

P-1026

Resistance of Attacin E transgenic lines of Royal Gala Apple to *Erwinia amylovora* (Fire Blight). H.S. ALDWINCKLE, J.L. Norelli, E. Borejsza-Wysocka, J.-P. Reynoird and M.V. Bhaskara Reddy. Dept. of Plant Pathology, Cornell University, Geneva, NY 14456. E-mail: hsa1@cornell.edu

The enterobacterium *Erwinia amylovora* causes the devastating fire blight disease of apples in many temperate regions. The immunoprotein attacin E from *Hyalophora cecropia* inhibits enterobacteria. Its encoding gene, *attE*, was transferred to Royal Gala (RG) apple by Agrobacterium-mediated transformation. In 1998, 2-yr-old plants of *attE*-transgenic lines growing in the field were artificially inoculated by bisecting the two youngest leaves of vigorously growing shoots with scissors dipped in 5 X 107 cfu of virulent *E. amylovora* strain Ea273 /ml. 8 wk after inoculation the length of necrotic lesion was determined as the % of the current season's shoot length (SLB) as a measure of disease. Several transgenic lines had significantly lower SLB than inoculated non-transgenic RG. Transgenic line TG138, containing *attE* under control of the Ppin2 promoter, had only 5% SLB compared with 56% SLB in non-transgenic RG. Western analysis indicated that TG138 had a high constitutive level of attacin expression. Northern analysis of in vitro material confirmed the constitutive expression of attacin in TG138, but indicated elevated attacin expression 1 hr following leaf wounding. When the field trial was repeated, many lines identified as resistant in 1998 also were resistant in 1999 tests: line TG138, most resistant of all lines tested in 1998, was again most resistant in 1999. A positive correlation seen between attacin content of Ppin2*attE* transgenic lines and fire blight resistance when growth chamber plants were inoculated with *E. amylovora* in 1998 was confirmed in 1999 field studies. Fruit of *attE* transgenic RG lines, which appeared indistinguishable from normal RG fruit, have been graded for size and color, pressure tested for firmness with and without skin, and assayed for soluble solids and titratable acidity. Data are currently being analyzed.

P-1027

Somatic Embryogenesis from Isolated Tissues of Mature Zygotic *Pinus palustris*. ALEX M. DINER. U.S.D.A. Forest Service, SFRC, Univ. FL, Gainesville, FL 32611. Email: ADINER@GNV.IFAS.UFL.EDU

Isolated hypocotyl and root apices as well as suspensors from mature, cotyledonary zygotic embryos of *Pinus palustris* were studied for somatic embryogenesis. Zygotic embryos were collected bi-weekly from 2nd.-yr. cones during 8/1–10/14, 1999. Hypocotyl apices, root tips and vestigial suspensors were cut from surrounding tissues under microscopy and removed to an agar-solidified 1/2-strength Brown and Lawrence medium containing glutamine (1mM), sucrose (0.1M), and casein hydrolysate (1%). Following dark-incubation at 20–22C, somatic embryogenic masses (SEM) were observed from all explant types. Frequency of SEM development was 7.2, 0.3 and 3.0 percent from hypocotyl apices, root tips and suspensors, respectively. SEM were uniquely visible from root tip explants within 1 week following excision from the embryo. Somatic embryos consisting of a single embryonal head cell and 2–3 suspensor cells were recorded. Cotyledonary somatic embryos were developed from the SEM from each explant type. A single instance of SEM development was realized in 1200 attempts using hypocotyl apices of embryos from 3-year stored seeds of longleaf pine. This process not only extends the temporal window-of-opportunity for SEM source-tissue collection from a few weeks in July to several later months, but also assures single-genotype embryogenic masses.

P-1028

Walnut Trees That Flower *In Vitro*: Micropropagation, Somatic Embryogenesis, Plant Regeneration, and Genetic Transformation. C. BRETON, D. Cornu, P. Capelli, D. Chriqui, L. Chrétien, E. Germain, and C. Jay-Allemand. Forest Tree Improvement, Genetic, and Physiology, I.N.R.A.-Orléans, 45166 Olivet Cedex, FRANCE. Email: breton@orleans.inra.fr

Some walnut trees (*Juglans regia*) growing in Central Asia (Uzbekistan, Tadzhikstan,. . .) are characterized by very early flowering capacities. These 'Early Mature Trees (EMT) may produce flowers as early as three months after germination. Thereafter, flowering remains very intense—up to three fruit sets per year—and each inflorescence may carry male, female, or both male and female flowers. The work presented here concerns the production of *in vitro* material and the transformation of these very peculiar trees. Walnuts obtained from an EMT collection orchard were used to generate somatic embryos and microshoot lines (Deng & Cornu. PCTOC (1992), 28:195–202 and Jay-Allemand, et al. Sci Hort (1992), 51:335–342) (lines 6.2, 11.4, and 53.6). Except for the line 6.2, plantlets could be generated through somatic embryo conversion and microshoot adventitious rooting. In our culture conditions, microshoot multiplication rates varied between 1.5 and 2 every three weeks. *In vitro* rooting averaged at 23% and 75% for the 11.4 and 53.6 lines, respectively. Interestingly, flower buds could be observed at the tip of the microshoots after 3 to 6 subcultures. Histological analysis of these structures revealed stamen primordia as well as orthotropous ovules. Most of the flowers that developed *in vitro* were hermaphrodite. Flower production will now be analyzed among the plants transferred in the greenhouse. The use of this developed *in vitro* model to study tree flowering abilities will be discussed. Genetic transformation and molecular analysis are in progress in order to pinpoint genes that could control juvenile induction among EMT genotypes.

P-1029

Increasing the Yield of Maturing Loblolly Pine Somatic Embryos Through Simultaneous Optimization of Cell Density and ABA Concentration. T.R. VALES & G.F. Peter. The Institute for Paper Science & Technology, Atlanta, GA 30318. Email: Gary.Peter@ipst.edu

Somatic embryogenesis is a promising technology for capturing and propagating genetically improved pine genotypes. While current methods for somatic embryogenesis can routinely produce maturing loblolly pine cotyledonary embryos, improvements are required in the quality and quantity of embryos produced before the process can be used on a large scale. In a recent set of experiments, early stage embryos were matured at different cell densities in combination with different amounts of ABA in the media and the number of early stage embryos that developed into cotyledonary embryos was determined. These two variables tested each have been shown to affect somatic embryo maturation in other plant species, however, these variables have never been optimized in combination for loblolly pine. The particular cell densities tested ranged from 1.0 ml to 0.06 ml settled cells per plate. The concentrations of ABA in the media that were tested ranged from 19.6 uM to 98 uM. Results from two different genotypes showed that a greater proportion of early stage embryos matured when the cell density was between 0.25 and 0.5 mls and with ABA concentrations in the media between 29 and 49 uM. Maturation at higher and lower densities was inhibitory. The concentration of ABA in the media necessary for optimal embryo maturation depended on the cell density. This data suggests that there is an optimal ratio of cell density to ABA concentration in the media that will maximize the percent of embryos that mature.

P-1030

Direct Somatic Embryogenesis through Pseudo-Bulblet Thin Cell Layer of *Lilium longiflorum*. DUONG TAN NHUT[1], Bui Van Le[2], Seiichi Fukai[3], Michio Tanaka[3], K. Tran Thanh Van[4]. [1] Institute of Biology in Dalat, 116 Xo Viet Nghe Tinh, Dalat City, Lam Dong, Vietnam; Present Address: Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa 761-0795, Japan; [2]Faculty of Biotechnology, Ho Chi Minh University, 277 Nguyen Van Cu, Ho Chi Minh City, Vietnam; [3]Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa 761-0795; [4]Institut de Biotechnologie des Plantes, UMR 0569, Universite de Paris-Sud, F-91405 Orsay Cedex, FRANCE. Email: duong@stmail.ag.kagawa-u.ac.jp

Lily is one of the most important bulbous crops and is used throughout the world in the flower industry as cut flowers and potted plants. The success of breeding programs of the *Lilium* species depends on having a procedure for producing plants rapidly. For this purpose an *in vitro* technique is frequently used. Such *in vitro* procedures are used increasingly for *in vitro* selection, protoplast fusion or genetic transformation as well as regenerating plants by somatic embryogenesis. Recently, somatic embryogenesis was shown to occur from bulb scales in some lily hybrids (Haensch 1996), and in *Lilium longiflorum* by using styles and flower pedicels (A. Tribulato et al., 1997), but in these procedures somatic embryogenesis was induced via callus culture. In the research now to be reported, somatic embryogenesis was achieved directly from pseudo-bulblet thin cell layer (TCL) of *Lilium longiflorum*. Embryo-like structures were obtained from different size explants of pseudo-bulblet TCL after 2 months of culture on Murashige and Skoog, 1962 (MS) medium containing 1 mg l^{-1} NAA and 0.5 mg l^{-1} TDZ. The embryo-like structures were then isolated and proliferated on MS medium containing 1mg l^{-1} NAA and 0.2 mg l^{-1} TDZ for their mass propagation every two months. The explant size of 0.8–1 mm in length was shown to be optimal for obtaining the highest number of embryo-like structures. For plant regeneration these structures were transferred to hormone-free MS medium with 30 g l^{-1} sucrose. All of these structures formed plantlets 3 months of culture.

P-1031

Image analysis: A Practical Approach for Evaluation of Soybean (*Glycine max* L. Merrill.) Somatic Embryo Growth. M.T. BUENROSTRO-NAVA[1], H.M. Frantz[1], P.P. Ling[2] and J.J. Finer[1]. [1]Department of Horticulture and Crop Science and [2]Department of Food, Agricultural, and Biological Engineering, The Ohio State University, Wooster, Ohio 44691, Email: buenrostro-nava.1@osu.edu

Image analysis can be used to collect valuable information on growth and development of plants and plant tissues over time. Computer vision has been used to monitor the kinetics of embryo formation and development, and allows precise measurement of the size, shape and color of the embryos. In this study, image collection, analysis and subsequent compilation of images to generate animations were explored to gain a better understanding of soybean somatic embryo development. Proliferative embryogenic tissue of soybean was transferred to development medium under two environmental conditions (23°C and 27°C). Digital images of the starting material and developing embryos were captured, stored, processed, and analyzed using Visilog and Adobe Photoshop software. Color was assigned to the embryos based on the CIE 1931 chromaticity diagram (x, y). Embryos grown under the two different environments, showed differences in the rate of growth and synchrony of embryo development. Although embryos growing at 27°C show a higher rate of growth than embryos growing at 23°C, animation clearly showed that the growth of the embryos at the higher temperature was asynchronous. These embryos also did not efficiently convert into whole plants. Color analysis indicated that the pale yellow (0.38, 0.36) embryogenic tissue had a lower capacity for differentiation compared to pale green (0.37, 0.62) embryogenic tissue, which showed a high capacity for differentiation. These results suggest that color of globular embryos may be a good parameter to estimate embryo differentiation and to predict embryo quality. Image analysis can provide a rapid, simple and complete descriptive summary of certain biological processes such as plant embryo growth and development. Use of image analysis for dynamic growth studies and combined with the gfp as reporter gene could provide a very powerful tool to analyze gene expression over time in intact cells, tissues or entire organisms.

P-1032

Isolation of genes expressed during somatic embryogenesis in orchardgrass. K. ALEXANDROVA and B.V. Conger. Department of Plant and Soil Sciences. The University of Tennessee. Knoxville, TN 37901-1071. E-mail: CONGERBV@UTK.EDU

Somatic embryogenesis has been studied extensively at the gross morphological level, but there is only a limited understanding about the molecular events taking place during this process. An efficient and repeatable system for somatic embryogenesis in orchardgrass (*Dactylis glomerata* L.) was used to study the molecular events taking place during this process. A highly embryogenic genotype Embryogen-P and a nonembryogenic genotype designated as Nonembryogen were used in these experiments. Segments from the basal 30 mm of the two innermost leaves were cultured on Schenk and Hildebrandt medium amended with 30 microM dicamba for induction of somatic embryogenesis. Eleven cDNA fragments that appeared only in leaf segments of Embryogen-P induced for embryogenesis were identified by differential display. The fragments were a product of PCR amplification with three one-base-anchored oligo-dT primers H-T_{11}G, H-T_{11}A and H-T_{11}C, and five arbitrary 13-mers. They were cloned in a pCR2.1 vector and sequenced. The cDNA fragments were from 191 to 342 bp in length and represented the 3' end of the transcripts. The entire transcripts were isolated from a cDNA library by using the fragments as hybridization probes.

P-1033

Development of Transgenic Peanut *(Arachis hypogaea L.)* Plants Producing an Edible Vaccine against Cholera. J. JACKSON, M. Egnin, H. Mason, and C. S. Prakash. Center for Plant Biotechnology Research, Tuskegee University, Tuskegee, AL 36088 (megnin@tusk.Edu; prakash@tusk.Edu

Every year, many in developing countries suffer and often die from enteric diseases caused by endemic bacterial pathogens, such as cholera. These diseases can be easily treated and prevented through effective vaccination and proper sanitation practices. However, costs to manufacture such life-saving vaccines can be expensive, especially for developing countries. Cholera, which is caused by the organism *Vibrio cholerae*, is an acute diarrheal disease, which produces symptoms that include watery diarrhea, vomiting, and severe muscle cramps. The development of transgenic plants to produce vaccines against infectious diseases such as cholera, is an inexpensive alternative to current systems for production of subunit antigens. In this study, transgenic peanut (*Arachis hypogaea* L.) plants, producing an edible vaccine against cholera were developed. The cholera synthetic LT-B (sLT-B) gene, under the control of the double CaMV $35S^2$ promoter, was inserted into the binary vectors, pGPTV-*HPT* and pGPTV-*KAN*. Synthetic LT-B (sLT-B) was introduced into peanut cultivar 'New Mexico' select (Valencia market type) via *Agrobacterium tumefaciens*-mediated transfer. The presence of sLT-B gene was confirmed through PCR analysis using sLT-B specific primers. Southern blot analysis further confirmed the stable integration of the cholera gene into the plant genome. Western blot analysis of proteins extracted from transgenic peanut plants was positive against cholera sLT-B specific antibody. The results of this study suggest the feasibility of genetically engineering peanut to produce and edible vaccine against cholera. However, futher studies will be needed to examine the immunogenicity of antigenic proteins produced in peanut.

P-1034

Durum Wheat with Altered Protein Expression Patterns Following Transformation with a High Molecular Weight Glutenin Gene. M.C. JORDAN, S. Cloutier, C. Rampitsch, and N. Ames. Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Rd., Winnipeg, MB, R3T 2M9 CANADA

The tetraploid durum wheat cultivar AC Morse (A and B genomes) was transformed via particle bombardment using a mixture of two independent plasmids. One construct contained the green fluorescent protein (GFP) gene under the control of the rice actin promoter while the other construct contained a high molecular weight glutenin gene (Dx5) from the D genome of hexaploid wheat under the control of the Bx7 promoter. Bx7 is a high molecular weight glutenin gene found in some genotypes of both hexaploid and durum wheat. Transformed shoots were selected on the basis of GFP expression. Co-transformation with both the GFP and glutenin genes was confirmed using Southern analysis of both T0 plants and T1 progeny. One transgenic plant was found to contain a large number of copies of the transgenes. The progeny of this plant not only did not show expression of the high molecular weight glutenin gene, but they exhibited suppression of expression of the entire high molecular weight glutenin fraction (multiple proteins encoded by a large gene family). Some progeny lines with almost complete suppression of the high molecular weight glutenin fraction were isolated. Another transgenic line exhibited a novel gene expression pattern in which altered expression of both high and low molecular weight glutenins was observed.

P-1035

Transgenic Cyclamen persicum Mill., produced from Etiolated Hypocotyls, stably express the gusA Reporter Gene in Petals, Scapes, Leaf Laminae, Petioles and Corms, 27 months after infection with Agrobacterium tumefaciens. M.R. BOASE, G. B. Spiller and T.A. Peters. New Zealand Institute for Crop & Food Research Limited, Private Bag 11-600, Palmerston North, New Zealand. Email:Boasem@crop.cri.nz

Gene transfer systems are an important prerequisite to molecular breeding of cyclamen. Transgenic cyclamen were produced using etiolated hypocotyls of cultivar Sierra Rose, grown in the dark for 63–101 days after sowing seed. Hypocotyl explants were precultured for three to five days in the dark on regeneration medium, then infected with either the disarmed Agrobacterium tumefaciens strain LBA4404, containing the binary vector pMOG410, or the disarmed strain EHA105, containing pART 27–10. Both binary vectors carry a nos-nptII gene construct and a 35S-gusA-intron. Explants were cocultivated with agrobacteria for four days on cocultivation medium containing 200μM acetosyringone. Transformed cells, selected using 50mg/l kanamycin, regenerated shoots via adventitious organogenesis in the presence of 1mg/l BAP and 1mg/l IAA. 106 transgenic shoots produced from three experiments were rooted in the presence of 100mg/l kanamycin and 0.1 mg/l NAA. 52 of these in vitro plantlets (49%) were positive for the b-glucuronidase (GUS) enzyme, when leaf laminae were assayed histochemically. Petals, scapes, leaf laminae, petioles and corms from ten independent transformants growing in a containment glasshouse were still GUS positive, 27 months after explants were infected. Northern analyses of leaf laminae confirmed expression of the gusA transgene.

P-1037

Comparison of Visual Screening, Herbicide and Antibiotic Selection of Transgenic Oat Plants. A.R. CARLSON and H.F. Kaeppler. Department of Agronomy, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706. Email: arcarlson@students.wisc.edu

Chemical selection of transgenic cells has been the preferred technique of plant transformation since its inception. The development of visual screening techniques provides an alternate method for producing transgenic plants. No direct comparison between visual screening and chemical selection has been performed to date. In this study herbicide, antibiotic, and visual screening of transgenic oat plants were compared. Embryogenic oat callus was microparticle bombarded and selection was performed using established protocols. A simple transformation vector containing the ubiquitin promoter and intron expression cassette with either bar (herbicide resistance), nptII (antibiotic resistance) or gfp (visual marker) genes was used for the comparison. The experiment was replicated seven times using four bombarded plates of each treatment per replication. In total, 128 calli were isolated using gfp-based visual screening, 111 calli using bar selection, and 86 calli using nptII selection. The number of visual screened calli that advanced to regeneration and remained fluorescent dropped to 40% of the original amount, as expected from previous studies. Most of the calli from bar and nptII selection made it to regeneration media, however there exists a potential for escapes at this point. The visual screening technique advanced events to the regeneration phase two weeks faster and involved less labor and materials to maintain. Results from molecular analysis and comparisons of transformation and regeneration efficiencies will be presented and discussed.

P-1038

Purine and Pyrimidine Metabolism During the Partial Drying Treatment and Germination of White Spruce (*Picea glauca*) Somatic embryos. C STASOLLA[a], N. Loukanina[a], I. Ashihara[b], E.C. Yeung[a], T.A. Thorpe[a]. [a]Plant Physiology Research Group, Department of Biological Sciences, University of Calgary, Calgary, Alberta, CANADA, T2N 1N4. [b]Department of Biology, Faculty of Science, Ochanomizu University, Tokyo, 112-8610, JAPAN. Email: cstasoll@ucalgary.ca

Changes in the pattern of purine and pyrimidine metabolism were investigated during the partial drying treatment (PDT) and the first days of white spruce somatic embryo germination. During the PDT, despite a decline in the total uptake of labeled precursors, a fast turnover of purine and pyrimidine nucleotides was observed. For purines, a large proportion of supplied adenine and adenosine was extensively salvaged by the enzymes adenine phosphoribosyltransferase (APTRase) and adenosine kinase (AKase) respectively, whereas inosine was degraded to CO_2 and ureids. For pyrimidines, uridine was the main substrate for the salvage pathway since a large proportion of uracil was catabolized as degradation products, i.e. CO_2 and ß-uriedopropionate. Uridine was mainly salvaged by uridine kinase, which increased as the embryos dried. Upon germination, a pronounced increase in the total uptake was observed for both purine and pyrimidine intermediates. For purines, the increment in specific activity of AKase and APRTase was mainly responsible for the large proportion of radioactivity from adenine and adenosine recovered into the nucleic acid and ATP+ADP fractions. For pyrimidines, the decline in the specific activity of uridine kinase was concomitant with a lower fraction of uridine being salvaged into nucleic acids and nucleotides. Conversely, as germination progressed, orotate phosphoribosyltransferase, a central enzyme of the pyrimidine de novo pathway, was found to increase and to contribute to a larger amount of orotate being anabolized. Taken together, these results indicate that the partial drying treatment represents an important transition for the enlargement of the purine and pyrimidine nucleotide pool required at the onset of germination, before the reactivation of the nucleotide de-novo biosynthesis.

VT-1001

Signal Transduction in Lymphocyte Locomotion: Microgravity Induced Lesions. A. SUNDARESAN[1], D. Risin[1] and N.R. Pellis[2]. Cellular Biotechnology Program, [1]Wyle Life Sciences and [2]NASA/Johnson Space Center, Houston, TX 77058. E-mail: alamelu.sundaresan1@jsc.nasa.gov

Adherence to, and locomotion through, interstitium is an important component of the immune response. Conditions such as true gravity (TG) and modeled microgravity (MMG) severely inhibit lymphocyte locomotion in vitro through gelled Type I collagen (Pellis *et al.*, 1994, 1997). We used the rotating-wall vessel bioreactor (RWV) as a prototype for modeled microgravity. Polyclonal activation of lymphocytes before exposure to MMG prevented locomotion inhibition. Phorbol myristate acetate (PMA) treatment of normal peripheral blood lymphocytes, after exposure to MMG, restored lymphocyte locomotion by 84%. Calcium ionophore alone or with PMA had no effect on MMG-exposed lymphocytes. However, direct activation of Protein Kinase C (PKC) with PMA restored MMG locomotion almost comparable to normal levels in lymphocytes cultured in static T flasks. Thus, events either at the level of PKC or upstream are affected by MMG. Treatment of lymphocytes with PMA and exposure to MMG in the RWV prevented loss of locomotion. Our model suggests that in MMG and TG lymphocytes experience severe loss of locomotion and the lesion is possibly a Ca^{++} independent isoform of PKC. Downregulation of PKC isoforms epsilon and delta was observed both at the messenger RNA and protein levels. Analysis of upstream events such as phospholipase C gamma expression was performed. Identifying the lesions in transduction pathways caused by exposure to microgravity. is essential to circumvent any immune system anomalies which might occur during long term space travel. Supported by NRA OLMSA-02 and NSCORT #NAG5–4072 grants.)

VT-1002

Transplantation of Mouse Pancreatic Islets Cultured in Microgravity-Stimulating Bioreactors. L. RUTZKY[1], M. Kloc[1], S. Bilinski[2], S. Stepkowski[1], T. Phan[1], H. Zhang[1], and S. Katz[1]. [1]Department of Surgery, The University of Texas Medical School, Houston, TX 77030 and [2]Institute of Zoology, Krakow University, Krakow, POLAND. Email: lrutzky@orgtx71.med.uth.tmc.edu

Culture of mouse pancreatic islets in HARV Rotating Wall Vessel (RWV) bioreactors preserves islet morphology and decreases immunogenicity of allogeneic islets. RWV bioreactors simulate some buoyancy effects of microgravity. Pancreatic islets were isolated form C57BL/10 (H-2^b) mice using collagenase digestion and discontinuous dextran gradient centrifugation. Freshly isolated islets or islets cultured in either stationary Petri dishes or bioreactors were transplanted under the kidney capsule of either syngeneic C57BL/10 or allogeneic C3H (H-2^k) streptozotocin-treated, diabetic mice. All allogeneic (n=17) and syngeneic (n=7) islets cultured either in dishes or bioreactors survived more than 100 days (p=0.001), as compared to fresh allogeneic transplants (mean survival time, MST=12±1.73 days, n=7). Nephrectomy of transplanted kidneys caused hyperglycemia, confirming that grafted islets maintained euglycemia. Immunostained fresh islets contained dendritic cells with strong Class II major histocompatibilty complex (MHC) expression. After 7 days of culture in either dishes or bioreactors, dendritic cells were absent, as confirmed by minimal expression of dendritic cell-associated antigens and Class II MHC and by electron microscopy examination. Dish-cultured islets showed signs of deterioration, with loss of nuclear heterochromatin and degenerating nucleoli, Golgi complexes, and secretory granules. In contrast, a and ß cells in bioreactor-cultured islets closely resembled fresh islets. In addition, large intercellular channels with intra-channel cytoplasmic processes were observed between certain arrays of bioreactor-cultured islet cells. The channels may help transport nutrients and gases into islets. Both fresh and bioreactor-cultured islets had large amounts of nuclear heterochromatin, well-developed nucleoli and Golgi complexes, narrow intercellular spaces, and well developed secretory granules. These studies indicate that simulated microgravity conditions maintain pancreatic islet morphology, while decreasing immunogenicity.

VT-1004

RNA Microarray Analysis of DFMO Induced Changes in Cancer-Related Gene Expression in Two Pre-cancerous Human Colon Polyp Cell Lines. E. ELMORE, R.A. Lubet, J.L. Redpath, G.J. Kelloff, and V.E. Steele. Department of Radiation Oncology, University of California, Irvine, CA 92697, Division of Cancer Prevention, National Cancer Institute, Bethesda, MD 20892. E-mail:eelmore@uci.edu.

Difluoromethylornithine (DFMO) has demonstrated chemopreventive efficacy in cell culture and animal models and is currently in clinical trials for the prevention of colon cancer. In our studies, changes in cancer-related gene expression in RNA microarrays was used to determine the effects of the treatment of two pre-cancerous colon polyp adenoma cell lines with clinically-relevant concentrations of DFMO (Meyskens et al., 1994. J. Natl. Cancer Inst., 86:112–1130.) VACO 330, which does not progress to cancer, and VACO 235, which does progress to cancer, were treated with three concentrations of DFMO, 3, 10, and 30 micrograms/mL. The exposure times included 0, 24, and 72 hours. RNA probes for forty-three cancer-related genes were used to make arrays on glass slides. Changes in RNA expression were determined by comparing the treated samples to the untreated control. In controls, genes such as c-jun, b-atf, mmp-3, and Cyclin D1 showed lower expression in VACO 235 cells compared to VACO 330 cells while apc, PCNA, jun-b, jnk-2 were higher. DFMO treatment resulted in a concentration-dependent inhibition of expression in many genes of both cell lines with concentrations of 3 and 10 micrograms/mL appearing to be the most active. Striking similarities in the response patterns of both cell lines as a function time and DFMO concentration were observed for several genes including erk-1, erk-1, c-fos, jun-b, and p53–5'. Data with the housekeeping genes gapdh and beta-actin suggest that these genes may be altered by DFMO treatment. The data show that DFMO at plasma concentrations found in clinical trials can alter cancer-related gene expression in pre-cancerous human colon polyp cell lines.

VT-1005

In Vitro Tissue Culture Models to Study the Interaction of Human Papillomavirus and Other Viruses of the Female Genitourinary System. C. Meyers, S. Alam, S. Andreansky, R.J. Courtney, M. Mane*, P.L. Hermonat*. Dept of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, PA and *Dept of Obstetrics and Gynecology, University of Arkansas Medical Center, Little Rock, AR.

The organotypic (raft) culture system has permitted the replication of multiple types of cervical cancer-associated human papillomaviruses (HPV). In vivo HPV typically infects the cervix in particular, but also infects other areas of the female genitourinary tract. HPV-positive biopsies are commonly coinfected with another infectious agent. Various epidemiological and experimental studies have reported the association of HPV and herpes simplex virus (HSV) in cervical lesions and in the development of cervical carcinoma. We report the use of the raft culture system to study the interaction of HPV and HSV. Raft cultures are the only reported system that can support the complete HPV and HSV replication cycles within the same tissue in vitro. We will report on our studies of the effect of this coinfection on the life cycles of both viruses and on the host tissue. Epidemiologically, HPV and adeno-associated viruses (AAV) are inversely associated with cervical cancer, with HPV causing and AAV protecting against this cancer. AAV, a helper dependent parvovirus, survives through its ability to interact with multiple virus types, including adenovirus, herpes simplex virus, and poxvirus. Furthermore, AAV has been shown to be a highly prevalent genital virus. These data have led us to the hypothesis that AAV's negative association with cervical cancer may be at least in part through its ability to inhibit HPV replication and their role in cervical carcinogenesis. AAV may have a protective effect against HPV-associated cervical cancer. We have begun investigations concerning interactions between AAV and HPV using the organotypic (raft) culture system capable of recapitulating the complete differentiation-specific replication cycle of HPVs including the synthesis of infectious virions.

VT-1006

Expression and Function of *Sonic hedgehog* and *Patched* Genes in Oral Epithelial Cells and Oral Squamous Cell Carcinoma Cell Lines. E. MICHIMUKAI, Y. TANAKA, N. KITAMURA, S. TORATANI and T. OKAMOTO. Dept. of Molecular Oral Medicine and Maxillofacial Surg. 1, Hiroshima University Faculty of Dentistry. Email: <eijim@ue.ipc.hiroshima-u.ac.jp>.

Patched gene encodes a transmembrane receptor protein with 12-membrane spanning domains and two large extracellular loops that bind their ligand, sonic hedgehog. The patched protein inhibits the sonic hedgehog signaling pathway by inhibiting the second cell surface protein, smoothened. Patched is important in determining the embryonic patterning and cell fate in multiple structures of the developing embryo. In addition, it has been considered that this signaling pathway plays a role in tumor progression. ★@We have studied expression and mutation of *patched* gene in DNA derived from oral squamous cell carcinoma (OSCC) cell lines (KA, KO, NA, NI, UE) and normal oral keratinocytes (HK) by RT-PCR, PCR-SSCP and direct sequencing. Furthermore, we have prepared a recombinant mouse sonic hedgehog-N (rmshh-N) and studied its effects on growth of OSCC cell lines and HK in serum-free culture. ★@PCR-SSCP analysis revealed that the PCR-single strand for exon 12 of *patched* from 4 OSCC cell lines (KA, KO, NI, UE) showed abnormally electrophoresed band. Direct sequencing of exon 12 of the 4 OSCC cell lines revealed a T to G transition in one allele at nucleotide 1682, indicating substitution Met to Arg at codon 561. Furthermore, we have observed growth stimulation by the rmshh-N on HK and NA that has no mutation in *patched*, but not on the other OSCC cell lines those have mutation in *patched*. ★@These results indicate that sonic hedgehog-patched signaling might have an important function in oral cancer progression.

VT-1007

Measurement of the Barrier Function of Cell Layers During a Metastatic Cell Challenge. C.R. KEESE, K. Bhawe, and I. Giaever, Applied BioPhysics, Inc. and Rensselaer Polytechnic Institute, Troy, NY 12180. E-mail: KEESE@BIOPHYSICS.COM

Electric Cell-substrate Impedance Sensing (ECIS) has been refined to report changes in the barrier function of confluent cells layers from impedance data. In this approach, cells are grown on small gold film electrodes and monitored using a weak AC current (usually 1 microampere). The electric current and small electric fields have no effect on the cells, but changes in the impedance of the system rendered by the cells can be used to identify alterations in cell morphology. We have employed these measurements to monitor the metastatic abilities of some cultured prostatic carcinoma cells of both murine and human origin in vitro. Human endothelial cells (HUVEC) were grown to confluence in the ECIS system and then exposed to the prostatic carcinoma cells. Data was collected at three different AC frequencies (namely 400, 4000, and 40,000 Hz) and processed with a model of the cell-electrode interactions to reports changes in (1) the barrier function of the HUVEC layer, (2) changes in membrane capacitance, and (3) alterations in the spacing between the HUVEC cells and the substratum. Results indicate that the initial response of the endothelial cell layer to the tumor cell challenge, in particular changes in barrier function, can be interpreted to indicate the degree of metastatic potential of the tumor cells. The value of this system over traditional filter TER measurements will be discussed. ——— I. Giaever and C.R. Keese, Nature 366, 591 (1993); C.M. Lo, C.R. Keese,and I. Giaever, Exp. Cell Res. 250, 576 (1999); Also see: http:\\www.biophysics.com

VT-1009

Involvement of Muscarinic Acetylcholine Receptor (mAchR) in Cytotoxicity of an Organophosphate Anticholinesterase, Diisopropylfluorophosphate (DFP). C.J. CAO*+, R.J. Mioduszewski *, D.E. Menking*, A.T. Eldefrawi+, and J.J. Valdes*. +Dept. of Pharm. & Exp. Therap., University of Maryland School of Medicine, 655 W. Baltimore Street, Baltimore, MD 21201; *US Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD 21010-5424. Email: james.valdes@sbccom.apgea.army.mil

Non-Cholinersterase mechanisms mediating actions of the anti-cholinesterase DFP were studied in Chinese hamster ovary (CHO)-K1 cells transfected with the gene encoding rat M1 or M3 mAchR and a human neuronal cell line. Ligand-receptor interactions were monitored in real time using a microphysiometer, the Cytosensor, which measured the rate of acidification of extracellular fluid as an index of cellular metabolic activity. Muscarinic agonists, carbachol or muscarine, induced a dose-dependent increase in metabolic activity in both cell lines, but DFP did not. When the CHO-K1 cells were pre-exposed briefly to DFP (1–1.5hrs), the agonist-evoked metabolic activity of the cells was significantly enhanced, but was inhibited by 24 hrs-exposure. A similar biphasic effect of DFP on mAchR in human neuronal cells was observed, but the inhibitory phase by DFP was more rapid than in CHO-K1 cells. Atropine (1 mu *M*), a nonselective antagonist of mAchR, not only completely blocked the receptor agonist-induced metabolic activity, but also completely inhibited the DFP enhancement of the responses in both CHO-K1 cells and human neurons. The data suggests that mAchR was involved in DFP action, in that the DFP changed the metabolic activity of these cells via the activation of mAchR.

VT-1010

Regulation of Differentiation Balance Between Osteogenesis and Adipogenesis by Gene Transfection. M. MIE, H. Ohgushi, Y. Yanagida, T. Haruyama, E. Kobatake and M. Aizawa. Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 226-8501, Japan. E-mail:mmie@bio.titech.ac.jp

In the case of osteoporosis or age-related osteopenia, the bone tissue looses its volume contrasting with the volume gain in the adipose tissue of marrow. The differentiation of osteoblast was found competitively balanced with that of adipocyte from bone marrow stromal stem cells which are potentiated in differentiating into osteoblast and adipocyte. These data suggest that the regulation of the balance between osteogenesis and adipogenesis is a key point of regulation in bone formation. We have applied a new strategy to modulate the balance between osteogenesis and adipogenesis by transfecting the gene encoding human bone morphogenetic protein-2 (hBMP-2) under the control of adipocyte specific lipoprotein lipase (LPL) promoter to the stromal stem cells. The transfected stromal stem cells should express hBMP-2, when the balance between osteogenesis and adipogenesis declines toward adipogenesis. Mouse embryonic fibroblast cell line C3H10T1/2 was transfected by BMP-2 gene. Cells were cultured in the presence of ascorbic acid and glycerophosphate. After a 14-day culture, osteogenesis was evaluated by measuring alkaline phosphatase (ALP) activity, along with adipogenesis evaluation by glycerol-3-phosphate dehydrogenase (GPDH) mRNA expression. In the presence of ascorbic acid, non-transfected cells expressed adipocyte phenotypes, accumulation of lipid droplets and increase of GPDH mRNA expression. But osteogenesis was not induced as assessed by ALP activity. On the other hands, in transfected cells, osteogenesis was induced by LPL-driven hBMP-2. And the expression of GPDH mRNA was lower than non-transfected cells. These results indicated that LPL-driven hBMP-2 acts to shunt C3H10T1/2 from adipogenesis to osteogensis.

VT-1011

Expansion of Murine Hematopoietic Progenitor Cells Population with Spatial Development of Stromal Cells in Porous Carriers without Cytokine Addition. M. TAKAGI, T. Sasaki and T. Yoshida. International Center for Biotechnology, Osaka University, 2-1, Yamada-oka, Suita, Osaka 565-0871, Japan. E-mail: takagi@icb.osaka-u.ac.jp

The spatial development of murine bone marrow cell culture using porous carriers was investigated in order to design a reactor possessing a three-dimensional hematopoietic microenvironment. Cells of a murine stromal cell line (SR-4987) could grow to a high density and spread well to form a fibroblast-like shape on a polyester nonwoven fiber disc (Fibracell (FC)), while those grown on porous cellulose beads formed a globular or elongated shape.[1)] Although the growth of primary stromal cells (attached cells) and the change in the total concentrations of hematopoietic cells (suspension cells) were similar for cultures of primary bone marrow cells employing these types of porous carriers without the addition of any cytokine, the proportion of progenitor cells (BFU-E, CFU-E, CFU-GM) in the total hematopoietic cell population increased only in the culture using FC. This three-dimensional microenvironment of a culture obtained using FC was associated with a marked increase in the proportion of macrophages and erythrocytes among mature cells that was not observed in Dexter's culture. The three-dimensional microenvironment of cells of a murine stromal cell line (ST2) on FC could increase the progenitor population of CFU-Mix more than two times during the culture of primary murine bone marrow cells, while the population markedly decreased on the layer of ST2 cells on the bottom of the dish. 1) Cytotechnology, 31, 225–231 (1999).

VT-1012

Growth Arrest Related eti-1 Gene (Epithelial Topoinhibition Inducible) with 6 RCC1 Repeats Induces Apoptosis. Masui,T.1, Iwashita,S.2, Takada,Y.1, Hayashi,M.1, and Mizusawa, H.1. 1 National Institute of Health Sciences, Setagaya-ku, Tokyo 158-8501, Japan, 2 Mitsubishi Kasei Institute for Life Science, Machida, Tokyo 194-8511, Japan. T. Masui e-mail: masui@nihs.go.jp

To understand the density arrest of normal human epithelium, we isolated growth arrest related (eti) genes by subtraction cloning of growing and density arrested epithelium (topoinhibition). The eti-1 gene produces two proteins, Eti-1 (309 aa) and delta Eti-1 (85 aa) by alternative splicing. Eti-1 has 6 RCC1 repeats and two potential glycosylation sites. RCC1 repeats were important in protein interactions of small GTPases. To examine the role of Eti-1, we have established HA-tagged loxp Adeno virus. Studies by glycosidase digestion and the deletion of potential glycosylation sites demonstrated that Eti-1 is a glycoprotein with molecular mass of 45 kd (40–50 kd), while delta Eti-1 is truncated just upstream of the glycosylation sites. We found that overexpression of Eti-1 or delta Eti-1 induced apoptosis of Vero cells and inhibited their colony formation. Interestingly Eti-1 was localized in perinuclear and on plasma membrane, while delta Eti-1 was observed in nucleus and nuclear membrane. We are now prepareing the antidody against Eti-1 to clarify the physiological role of enodogenous Eti-1 and delta Eti-1 in density arrest of human epithelium.

VT-1013

Disorder of Intracellular Localization of S100C in the Process of Immortalization of Normal Human Fibroblasts. M. SAKAGUCHI and M. Namba. Department of Cell Biology, Institute of Cellular and Molecular Biology, Okayama University Medical School, Okayama 700-8558, JAPAN. Email: mnamba@med.okayama-u.ac.jp

We investigated proteins down-regulated in immortalized human cells by 2-dimensional gel electrophoresis. As a result, S100C, a Ca^{2+}-binding protein, was dramatically down-regulated in immortalized human fibroblasts compared with their normal counterparts. Immunoflourescence staining of S100C showed that S100C was localized at the leading edge of F-actin in normal and immortalized fibroblasts at a low cell density. At confluence, S100C moved to and accumulated in the nuclei of normal cells, while in immortalized cells it remained in the cytoplasm. Microinjection of the anti-S100C antibody into normal confluent quiescent cells induced DNA synthesis. Furthermore, when exogenous S100C was compelled to localize in the nuclei of HeLa cells, their DNA synthesis was remarkably inhibited with increase in cyclin-dependent kinase inhibitors such as $p16^{Ink4a}$ and $p21^{Waf1}$. These data indicate the possible involvement of nuclear S100C in the contact inhibition of cell growth.

VT-1015

Cross-Contaminations of Human Leukemia-Lymphoma Cell Lines. Y. MATSUO[1], C. Nishizaki[1], W.G. Dirks[2], S. Habig[2], and H.G. Drexler[2]. [1]Fujisaki Cell Center, Hayashibara, Okayama, Japan; [2]DSMZ-German Collection of Microorganisms and Cell Cultures, Department of Human and Animal Cell Cultures, Braunschweig, Germany. E-mail: yomatsuo@hayashibara.co.jp

There are three major problems in the culture of continuous leukemia-lymphoma (LL) cell lines: (1) cross-contamination with other cells; (2) mycoplasma contamination; (3) availability of cell lines. In a collaborative study between two large LL cell line collections, we addressed the first point by DNA fingerprinting, screening a large panel of such cell lines in order to identify the exact frequency of cross-contaminated lines, the sources of false cell lines, and which cell lines are the main contaminators. Highly polymorphic loci, the alleles of which are the result of variable tandem repeat sequences (VNTRs), are informative genetic markers for DNA fingerprinting. Several suitable multilocus and single locus with VNTR sequences are known. Multilocus probes detect short tandem repeats spread throughout the genome while single locus probes detect one genomic spot, but both alleles. We used here PCR for amplification of human minisatellite loci (= amplified fragment length polymorphism), in combination with the restriction fragment length polymorphism analysis of the (GTG)5 multilocus. The VNTRs of the single loci D2S44, D1S80, D17S5 and ApoB1 span repeated DNA elements from 168–4000 bp. PCR gel data were digitized and entered into a specific databases; cell line HELA was used as an internal standard. Similar/identical PCR banding patterns were confirmed or excluded in a subsequent (GTG)5 multilocus analysis. We examined 315 cell lines, 207 were obtained from the original investigator, 108 from a secondary source; in 33 cases, the same cell line was obtained from more than one source. We found that 18%, 20% and 14% of the cell lines overall, from original sources, and from secondary sources, respectively, were cross-contaminated. The most commonly detected contaminating cell lines were: CCRF-CEM, HL-60, Jurkat, K-562, Nalm-6, Reh, and U-937. The sequential use of single locus and multilocus fingerprinting provides an extraordinarily high exclusion rate for false positive/negative data.

VT-1027

Inflammatory Activation of Neutrophils Triggered by a Novel Class of Peptides. H. MUKAI[1,2], Y. Hokari[2], T. Seki[2], H. Nakano[2], T. Takao[3], Y. Shimonishi[3], E. Munekata[2] and Y. Nishi[1]. [1]Lab. of Life Sci. & Biomol. Eng., Japan Tabacco, Inc., Aoba-ku, Yokohama, Kanagawa 227-8512, Japan, [2]Inst. of Applied Biochem., Univ. of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan, [3]Inst. of Protein Res., Osaka Univ., Suita, Osaka 565-0871, Japan. Email: hidehito.mukai@ims.jti.co.jp

Neutrophil is one of granulocytes that has important roles to protect against not only infection but also tissue injury. They quickly infiltrate to infection or injury sites, produce superoxide and digestive enzymes, and phagocytose infected microorganisms and cell debris. They also produce various inflammatory cytokines and cause the inflammatory reactions. Most of characterized CXC chemokines such as interleukin 8 that activate neutrophils are produced and secreted after the initial inflammatory reactions occur, suggesting the presence of neutrophil activating substances to promote rapid migration. In this study, we purified a novel class of neutrophil activating peptides. Several peptides were isolated from healthy porcine hearts and determined their structures. All of them were the fragment peptides of cytosolic proteins. Two peptides among them chemically synthesized had activities to stimulate chemotaxis and secretion of digestive enzymes from neutrophils.We propose a novel mechanism to induce initial inflammation based on present results; some of the fragment peptides of cytosolic proteins leaking from damaged cells cause the activation of neutrophils to scavenge toxic debris at the inflammation sites.

E-2000

Training for the Future: Cell Culture and Biosafety Workshops. MARGARET L. SMITH and Brenda J. Wong. CORE Cell Culture Facility and Environmental Health and Safety, University of California, San Diego, CA 92093-0605. E-mail: M2Smith@ucsd.edu.

Now that many biological vectors and molecular reagents are readily available from commercial sources, investigators who have little training in virology or cell culture techniques are initiating research utilizing gene transfer. This comes at a time when these techniques are under increased scrutiny by the public and government regulations regarding these materials are increasing. In the USA, many new enforcement and training responsibilities for biosafety are being passed directly to universities and principle investigators. In response to this need UCSD has developed a series of professional training workshops emphasizing appropriate biosafety and aseptic techniques. We believe that use of good laboratory practice and aseptic technique also provides the highest level of biosafety, particularly in our rapidly changing biomedical research environment. A basic cell culture workshop was begun this fall and modules are planned covering advanced procedures. The material presented is very practical and all techniques are repeated in lab exercises under close supervision. The workshops are jointly funded by donations of products from companies and from participant fees. A workshop manual and bench protocols have been developed. We will present an analysis of these workshops, their content, instructional materials and student feed back. Each workshop group conducts a small experiment optimizing some parameter of the technique; these class results will be presented. We hope to hear of the experience of other members of the society and to promote an exchange of ideas for future training in biosafety and cell culture.

E-2001

Affordable Plant Tissue Culture for the Classroom. CAROL STIFF, Kitchen Culture Kits, Inc., Olympia, WA. Email:KCK@TURBONET.COM Janet Clancy, Washington State University and Pullman High School, Ann Evancoe, Hudson Valley Community College, Troy, NY and Colleen Fiegel, Ben Franklin High School, New Orleans, LA.

Plant tissue culture can be used effectively to stimulate interest in science in the elementary, junior, senior, and post-secondary school classrooms. Students learn about plant growth, morphology, anatomy, effects of different plant hormones, interaction of microorganisms, and the importance of sterile technique. Use of plant tissue culture has been limited in the past due to the need for expensive equipment (laminar flow hood, autoclave). However, through the use of biocides, such as PPM, expensive equipment is no longer essential for the successful completion of these experiments. Using inexpensive household supplies (microwave, baby food jars, bleach, plastic storage box, etc.), plant tissue culture experiments can be conducted in the classroom or home with little problem of contamination. A description of these methods, and reports of successful completion of experiments, will be presented.

E-2002

DNA Isolation—A Comparison of Two Methods for the Secondary School Science Classroom. A.KEPPING and J. HSIEH

Traditionally, DNA isolation requires the use of hazardous solvents. This is unacceptable for the secondary science classroom. The purpose of this experiment is to compare two protocols for the isolation of DNA from *Drosophila melanogaster* that do not require hazardous substances. The first method is a pre-packaged universal DNA extraction kit tht can be purchased commercially. No information regarding the reagents in this kit is supplied with it. The second method utilizes InstaGene Matrix™ method. Additional product information, including how the matrix isolates DNA is known. Both methods isolate DNA effectively. The kit uses RNAase to remove RNA and therefore a discrete DNA band is visible after electrophoresis and staining. The disadvantage of the kit is that it requires many more stepts than the InstaGene Matrix™ method. The matrix method is easier and faster to do, but since RNA is not removed, the nucleic acids appear as a 'smear' rather than as a discrete band. We compared the products from each isolation for suitablity in a PCR reaction. We used primers that other students had designed to target *Drosophila melanogaster* genes. We found that both protocols are equally suited for use in PCR. Since the matrix method is so much easier to to, we recommend it rather than the kit for classroom use.

E-2003

LIGASE Science Education Program: Teacher Training and In-house Research Programs. Zuzana Zachar. Department of Biochemistry and Cell Biology, SUNY, Stony Brook, NY 11794-5215 E-mail: zzachar@ms.cc.sunysb.edu

LIGASE is a multifaceted science education program. One of its major components is teacher education and training. We have developed graduate courses in biology specifically designed for teachers of secondary and middle school students. The courses combine lectures on current topics in biotechnology, genetics, microbiology and neurobiology with intense hands-on laboratory training in techniques appropriate for transfer into the secondary or middle school science classroom. We also recruit teachers as partners in running our various summer science camps for middle and secondary school students and the junior college students bioprep program. Many of the teachers who have participated in our program have gone on to initiate and run successful in-house research programs in their home schools. This expands considerably the opportunities in which secondary school students can learn about and do research.

E-2004

Investigation of Evolutionary Relationships Among Mammals Using the Polymerase Chain Reaction. Y.HAN, P. FRAKE, L. PALUSKA. Northport High School, Northport, New York

Evolution may be defined as the change in the genetic composition of a population over time. The process of evolution can be studied by examining the fossil record and biogeography. It can also be studied by comparing anatomical, physiological, and embryological homologies. With the advent of modern biochemical technology, it has become possible to study the evolution of species this way as well. In these experiments, we sought to examine various orders of mammals to see if we could detect homology with respect to the possession of variable number tandem repeat regions found on human chromosomes number 1, 16 and 17. Our expectation was that certain orders of mammals such as the primates we tested might have all three regions and that others (hedgehog) might have none. We found that all three human primers found a match with DNA from the chimpanzee, but no matches were found with DNA from a baboon. We do not believe non-specific binding of primers occurred because calculated base pairs for each DNA band were within the allowed PCR product size range. A more likely explanation is that some hair samples simply did not contain follicular DNA.

E-2005

Chemically Modified Tetracycline 300 Inhibition of PMN Lysate Mediated Degradation of Alpha 1-Protease Inhibitor (alpha1-PI) LINDSEY PALUSKA. Northport High School, Northport, New York, 11768

Pathophysiological processes such as emphysema, cystic fibrosis, and even tumor cell metastasis occur when proteinase activity exceeds the protective capacity of endogenous inhibitors. Polymorphonuclear cells (PMNs or neutrophils) release a variety of proteinases such as human neutrophil elastase (HNE) and matrix metalloproteinases (MMPs) at the inflammatory site, which leads to connective tissue degradation. Human alpha 1-protease inhibitor is the predominant endogenous inhibitor of HNE that protects against elastic fiber degradation. This protease/antiprotease balance is further complicated by the fact that MMPs proteolytically inactivate alpha 1-PI, resulting in increased tissue damage. Chemically modified tetracyclines may have potential therateutic applications in the management of the above diseases because of their ability to inhibit HNE and MMPs. CMT 300 was studied in vitro for possible therapeutic efficacy as an inhibitor of PMN lysate mediated degradation of alpha 1-PI. PMN lysates were obtained by processing neutrophils, which yields a milieu of enzymes, including HNE and MMPs. Varying concentrations of CMT 300 were incubated with alpha 1-PI and PMN lysate at 37 ^{0}C for 24 hours. The inhibition of PMN lysate mediated degradation of alpha 1-PI was measured using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). It was shown that CMT 300 prevented the degradation of alpha 1-PI in a dose dependent manner, such that lower concentrations of CMT 300 was a more effective inhibitor of alpha 1-PI degradation than higher concentrations. These results suggest that CMT 300 may serve as an effective exogenous inhibitor of the enzymatic degradation of connective tissue that occurs during the inflammatory response.

E-2006

The Effect Gastrointestinal pH on Chemically Modified Tetracyclines, An In Vitro Analysis of Potential Methods of Drug Delivery. P.C. FRAKE and E.Roemer and S. Simon. Department of Pathology, SUNY Stony Brook, Stony Brook, NY 11768

Adult respiratory distress syndrome, cystic fibrosis, rheumatoid arthritis,and the metastasis of cancer tumor cells all involve connective tissue damage due to excess proteolytic activity. Chemically modified tetracyclines (CMTs) may have potential therapeutic applications in the management of these diseases due to their abilities to inhibit human leukocyte elastase (HLE) and matrix metalloproteinases (MMPs), proteases associated with inflammation and neoplastic invasiveness. Selected CMTs were studied in vitro to examine the effects of pH on their inhibitory properties. CMTs were incubated at body temperature at extreme pHs for given periods of time, to simulate exposure to acidic gastric secretions and alkaline pancreatic secretions, and then assayed at neutral pH to simulate the environment of the jejunum and ileum. CMT 300 (6-dimethyl-6-deoxy-4-de(dimethylamino) tetracycline)was compared to its derivatives 303, 306, 308, 315, 1001 and 1002. Dose dependent inhibition of HLE activity was observed with most of these CMTs at all pHs. However, after incubation at low pH (2) CMT 303's ability to inhibit HLE was completely eliminated. This elimination of HLE inhibitory activity was then proven to be due to a chemical change in the CMT 303 molecule by direct analysis of optical density. Thus, 303 would lose its potential therateutic effect after interaction with the pH of the stomach. Therefore, an enteric pill coating would need to be used to deliver the drug to the high pH of duodenum. Based on these data, the feasibility of enterically coating CMT 303 will be pursued before employing it in clinical trials.

E-2007

Cloning and Analysis of the Heart-Inducing Tbx5 Transcription Factor Gene from the *Xenopus tropicalis* Genome. Y. HAN. Northport High School, Northport, NY 11768

The gene Tbx5 is essential for heart development in *Xenopus laevis*, and mutations in the gene lead to human Holt-Oram syndrome. Information about its specific role in heart development can be found by examining its upstream regulatory sequences. Because the tetraploid *Xenopus laevis* is unsuitable for molecular studies, we identified and isolated Tbx5 in the genomic library of the diploid *Xenopus tropicalis* a closely related species. We are now sequencing the promoter region for analysis.

E-2008

Propagation of Daylily by Conventional and Non-conventional Methods. T.SWANN*,J.Carter and S.Dhir. *Houston County High School, Fort Valley State University, Fort Valley, GA 31030. E-mail:dhirs@mail.fvsu.edu

Everyday gardeners as well as commercial daylily growers need to know how to produce large number of new plants from their original stock in the least possible time. To answer this question, a study was conducted to compare two conventional propagation methods—drenching and soaking. Two hundred ramets of *Hemerocallis* cv. Stella de Oro, 10–15 mm in size at crown were used. The experiment consisted of: Drenching method—roots of ramets were exposed to different concentration of benzyl adenine (BA) by drenching the surrounding soil, Soaking method—bare rooted plants were soaked in BA solution, and a Control group—no treatment. After 34 days, the number of offsets was counted in each group. Irrespective of the method used, treatment of ramets with benzyl adenine solution produced more offsets than the control group. Highest number of offsets were counted with the soaking method. Thus, soaking is better than drenching with benzyl adenine for faster propagation of daylily, *Hemerocallis* cv. Stella de Oro. Experiments aimed at propagating daylily using non-conventional methods involved use of tissue culture methods. Young flower buds were collected from garden and surface sterilized before removing explants. Filament explants were cultured on MS+BAP 3.0 mg/l+IAA 0.5 mg/l medium. In 4 weeks, small pale yellowish embryos were formed. This confirms that daylily, *Hemerocallis* cv. Stella de Oro can be propagated at high frequency by tissue culture methods.

E-2009

Introduction of Vaccine Genes in Tomato. M.SHAH*, S.K. Dhir. *Warner Robins High School, Agricultural Research Station, Fort Valley State University, Fort Valley, GA−31030. E-mail:dhirs0@mail.fvsu.edu

Biotechnology has provided new strategies for the production of human vaccines in plants. Due to the high cost of vaccines extraction from *E. coli* using bioreactors, plants such as tobacco have been used for the production of vaccines. Advantages of plant based oral vaccines include low cost of extraction, improved reliability and safety, elimination of the need for injections, and availability in developing nations. The objective of the present study is to introduce and express the vaccine gene in tomato. Tomato containing vaccines could be eaten as raw food material, are inexpensive to produce, and are easily available in developing nations. In the first stage of the project, the aim was to develop a high frequency plant regeneration in tomato. Various concentration of the phyto hormones benzyl adenine (BA) and zeatin (ZT) were tested to determine their effect on the regeneration response of the tomato explants. Four different explants, the shoot tip, cotyledon, hypocotyl, and hypocotyl base were tested. Maximum number of shoots from all the four explants were observed in MS medium with BA 1.5 mg/l and ZT 0.5 mg/l. The explants with the maximum average number of shoots was the hypocotyl base. Natural sensitivity of different explant to selective agents: kanamycin, paromomycin, spectinomycin and streptomycin were also determined. Data on optimization of conditions for DNA delivery using particle bombardment will also be presented.

E-2010

Initial Assessment of the Effects of Battery Dumping in the Tennessee River; Results from Lead and Mercury Testing in River Invertebrates. TESA HARRIS. Giles County High School, Pulaski, TN 38478. E-mail: terrace@usit.net

Do batteries dumped into the Tennessee River leak lead and/or mercury into the sediment and pose any threat to the environment? Due to human activity in the Tennessee River, these contaminants are possible. In particular, the dumping of old batteries from navigational lighting has been commonplace. River invertebrates were taken out of the sediment at Hobbs Island, Alabama, near a possible source of contamination (navigational waterways). Three particular species were selected due to availability from a similar area well away. From the potential for battery dumping, a control sample was taken. The study was based on a model called The Mussel Watch Project, conducted by the US Department of Commerce, NOAA, to measure concentrations of lead (Pb) and Mercury (Hg) in whole soft parts of river invertebrates. Test performed on the invertebrate tissue were conducted according to a document by the EPA entitled, Test Methods for Evaluating Solid Waste, method 200.3, Sample Preparation Procedures for Spectrochemical Determination of Total Recoverable Elements in Biological Tissues. The tissue of the invertebrates was dissolved in up to 5 gr. of nitric acid, hydrogen peroxide and heat. This digestion results in a clear solution that was then analyzed by mass atomic spectrometry methods. Results demonstrated no noticeable contaminants. Further study with more sampling is underway.

E-2011

Mentoring High School Students in a Research Setting. S.DHIR. Plant Science, Fort Valley State University, Fort Valley, GA-31030. E-mail: dhirs0@mail.fvsu.edu

At Fort Valley State University (FVSU), we offer enrichment activities for high school students under the umbrella of a structured program, Summer Research Apprenticeship Program (SRAP). This program is aimed to enhance the awareness of the biological, agricultural and food sciences among minority high school students and encourage them to consider career opportunities in these fields. Since 1980, nearly 375 students from across Georgia have participated in the program, which lasts for seven weeks during summer at the FVSU campus. During their stay, apprentices are given instructions in cell biology, biotechnology, computer technology and agricultural economics. Supervised by faculty mentors, they use state-of-the-art equipment to get hands-on experiences. Filed trips to educational institutions and seminars by scientists from academia, industry and government organization form an integral part of the program. At the end of the program, the apprentices submit a written technical report and make an electronic presentation on his or her research activities to the faculty and staff. Details of the program activities will be presented. In addition, throughout the year, selected number of high school students are offered mentorship for research projects for science fair competition. Both these programs have shown a direct impact on student recruitment and retention.

I-2000

Down-Regulation of the Polyhedrin Gene by the Replication of an NPV and a GV in the Same Cell. G.F. CAPUTO, S.S.Sohi and B. M. Arif. Great Lakes Forestry Centre, Sault Ste Marie, ON, Canada. P6A 5M7. E-mail: gcaputo@nrcan.gc.ca

The effect of mixed infection with an NPV from *Choristoneura fumiferana* and a GV from *Choristoneura occidentalis* was investigated in larvae of the eastern spruce budworm. No obvious enhancement of NPV by GV was observed; a phenomenon that has previously been reported with some GVs that carry the enhancin gene. Light and electron microscopic observations of the mixed infection showed that both viruses replicated in the same larva. It was also observed that occasionally both viruses multiplied in the same cells; NPV in the nucleus and GV in the cytoplasm. In these dually infected cells, neither occlusion of NPV nor the synthesis of polyhedrin was observed. In contrast, the synthesis of granulin and the occlusion of GV were not affected. As well, in cells containing only one virus (in both singly and dually infected larvae) the normal occlusion process occurred. It clearly appears that in dually infected cells, GV either down regulated the expression of polyhedrin or interfered with the condensation and occlusion process. It was also observed that in dually infected larvae, aberrant GV capsules were observed and often the capsules were in isolation membranes within the infected cell. Polyhedrin was sometimes seen in autophagic bodies. Both of these events are normally associated with digestion of the materials within the isolation membranes or the autophagic bodies.

P-2000

Programmed cell death in fungi: Heterokaryon incompatibility involves nuclear DNA degradation. S.M. MAREK (1), J. Wu (4), N.L. Glass (3), D.G. Gilchrist (1,2), R.M. Bostock (1,2). (1)Plant Pathology, UC, Davis, CA 95616; (2)Center for Engineering Plants for Resistance Against Pathogens (CEPRAP), UC, Davis, CA 95616; (3) Plant and Microbial Biology, UC, Berkeley, CA 94720; (4)Biotechnology Laboratory, University of British Columbia, Vancouver, BC V6T 1Z3. E-mail: smmarek@ucdavis.edu

Within species, fungi can fuse to form heterokaryotic mycelia if they possess identical heterokaryon incompatibility (*het*) loci. If fungi attempting heterokaryosis differ at one or more *het* loci, the fused cells self-destruct by a killing reaction process reminiscent of apoptosis in animal cells. In *Neurospora crassa*, ten such *het* loci have been identified in addition to the mating locus. The *het-c* locus encodes a single nonessential protein and controls specificity as one of three alleles, *OR*, *PA*, or *GR*. In this study, the cytology of transformants carrying incompatible and compatible combinations of *het-c* alleles and pairings between vector control transformants were examined using fluorescent DNA stains and terminal deoxynucleotidyl transferase-mediated dUTP-X nick end labeling (TUNEL). Transformants carrying incompatible *het-c* transgenes were inhibited in growth and contained heavily degraded nuclear DNA in cells also displaying vacuolated or shrunken cytoplasms. Transformants carrying compatible *het-c* transgenes contained little or no degraded DNA. In pairings between incompatible control transformants, fusion cells frequently contained heavily degraded nuclei in disorganized cytoplasms. In pairings between compatible control transformants, fused cells rarely contained degraded nuclei. Cells killed by the incompatibility reaction often contained cytoplasmic and nuclear remnants that persisted after adjacent cells grew new hyphae into or around the killed cells. The process by which heterokaryon incompatibility kills and disassembles cells and the extent to which it is genetically controlled can best be described as programmed cell death.

P-2001

Role of Retinoblastoma-Related Protein in Programmed Cell Death in Diseased Plants. P.J. HOEGGER, J.M. Li, J.E. Lincoln and D.G. Gilchrist. CEPRAP, UC Davis, Davis, CA 95616. Email: dggilchrist@ucdavis.edu

Several lines of evidence suggest that the mechanisms that control the cell cycle and pro-grammed cell death (PCD) in animals and plants are similar. Recently, the existence of retino-blastoma (Rb)-related protein and other components of the cell cycle controlling apparatus was shown in plants. Furthermore, there also appears to be a dependant relationship between apop-tosis and cell cycle regulating factors. As in animals, PCD plays an important role in plant dis-ease. Early markers for PCD and characterization of the genes involved are needed to under-stand the mechanisms by which pathogens induce or suppress cell death to their advantage. Rb is a promising candidate for this purpose. In animals, Rb operates at the restriction point of the cell cycle and thereby controls the passage of cells through G1 into S phase. This passage is blocked by hypophosphorylated Rb, which physically associates with transcription factors of the E2F family. Hyperphosphorylation of Rb by various cyclin-cyclin dependant kinase complexes leads to disruption of the Rb-transcription factor association. Accumulating evidence suggests that Rb plays also a role in apoptosis. In studies with mammalian cells, results indicate that Rb is capable of suppressing apoptosis induced by interferon, transforming growth factor and the tumor suppressor p53. Furthermore, cleavage of Rb by caspases appears to be required for tumor ne-crosis factor-induced apoptosis. We have isolated an Rb-related gene from tomato and recent results on its role in PCD in diseased plants will be presented.

P-2002

Insect Resistant Tropical Maize Developed through the Introduction of a Fully Modified *Bacillus thuringiensis cry*IB Gene. BOHOROVA, N. [1], R. Frutos [2], M. Royer [2], N. Lecointe [2], P. Estañol [1], M. Pacheco [1], S. McLean [1], D. Hoisington [1]. [1] CIMMYT Int., Apdo. Postal 6-641, 06600 Mexico D.F., Mexico, E mail: n.bohorova@cgiar.org ; [2] CIRAD, BP 5035, 34032 Montpellier cedex 1, France

A synthetic version of the *Bacillus thuringiensis cry*IB gene was used in the transformation of tropical maize germplasm to confer resistance to major lepidopteran insect pests for tropical maize. The modified *cry*IB gene was synthetized using a procedure based on recursive principles. The sequence of the wild type *cry*IB gene was fully modified from nucleotide 1 to 1941, which corresponds to the sequence coding for the active toxin plus 29 amino acids on the N-terminal end. The G+C content of the modified gene is 58% while that of the original gene is 39.3 %. To generate transgenic plants, methods and protocols were established for biolistic bombardment, selection, and regeneration of immature embryos and calli from CIMMYT tropical lines and hybrids. Plasmids used contained the synthetic *cry*IB gene under the control of a maize ubiquitin or a rice actin-1 promoter and the selectable *bar* gene (PTT gene, Hoesht), under the control of the cauliflower mosaic virus (CaMV) 35S promoter, jointly present on a single plasmid. A total of 245 transgenic plants have been selected in T0 by screening for resistance to Basta ™; most of transgenics were further confirmed for the presence/ absence of the introduced *cry*IB gene by DNA hybridization using Southern blot analyses. Sixty of the 245 transformed plants that were herbicide resistant, displayed resistance to southwestern corn borer (SWCB) larvae, as determined by simple insect bioassay. In T1-T4, the transformed plants presented resistance to SWCB larvae with expected Mendelian segregation (1:1). Analyses of the progenies to confirm the sexual transmission of two introduced genes and their stable expression, with an emphasis on plants presenting resistance to SWCB, are ongoing.

P-2003

Development and Field-Testing of Walnut Trees Expressing the cryIA(c) Gene for Lepidopteran Insect Resistance. C.A. LESLIE [1], G.H. McGranahan[1], S.L. Uratsu[1], J.S. Tebbets[2], P.V. Vail[2], and A.M. Dandekar[1]. [1] Department of Pomology, University of California, 1 Shields Ave., Davis, CA 95616, [2] Horticulture Crops Research Laboratory, USDA, ARS, 2021 South Peach Ave., Fresno, CA 93727. E-mail: caleslie@ucdavis.edu

Walnut trees expressing the CryIA(c) insecticidal protein from *Bacillus thuringiensis* were generated from somatic embryos and planted in the field. A modified cryIA(c) gene was inserted into several genotypes including the commercial cultivar 'Chandler'. Somatic embryo lines expressing cryIA(c) under the regulation of two separate promoters, CaMV35S and ubi3, were recovered. The efficacy of these promoters was compared in bioassays using somatic embryos and first instar codling moth (*Cydia pomonella*) larvae. Statistical analysis showed clonal lines fell into three classes. Class A lines prevented insect development beyond first instar and produced 90–100% mortality. Class B lines allowed marginal development with larvae remaining at 1st or 2nd instar and mortality of 60 to 89%. Class C lines were indistinguishable from controls, permitting normal development to 2nd and 3rd instar and producing 0 to 59% mortality. Production of the CryIA(c) protein was confirmed and quantified by Western analysis. Somatic embryos of both high and low expressing clones were germinated, grafted to seedling rootstocks, and planted in the field. More than 200 trees were established at two orchard sites. Although promoter activity did not differ in somatic embryos, distinct differences were observed in field-grown leaves. Young and mature leaves in which cryIA(c) was regulated by CaMV35S produced larval mortality of 83% and 67% respectively. Under the regulation of ubi3, mortality was only 47% and 7%. Mortality was 4% and 0% in controls. In preliminary trials, both promoters produced elevated insect mortality in hull and kernel tissues.

P-2004

Transformation of Leaf Rust Susceptible Lines of Maize and Wheat with the Maize Rp1-D Rust Resistance Gene. M. STEINAU, S.H. Hulbert, and H.N. Trick. Department of Plant Pathology, Kansas State University. Manhattan, Kansas 66506. Email: steinau@plantpath.ksu.edu

Rusts are one of the most economically damaging pathogens on cereals worldwide. Yield loss in Kansas from wheat leaf rust in epidemic years has reached high as 11.3%. Additionally, new wheat cultivars with improved rust resistance currently have a life expectancy of about five years before virulent strains arise from mutations or by immigration typically minimize their effectiveness. Resistance genes transferred from sexually incompatible species could offer additional genes to combat fungal infestations. Recently, the *Rp1-D* gene from maize has been identified and cloned into pUC-based transformation vectors. To confirm the functionality of the *Rp1-D* gene, we have tranformed rust susceptible maize callus-lines with both genomic clone and an ubiquitin driven clone to overexpress the *Rp1-D* gene product. Several independent clones have been regenerated and these clones have confirmed by molecular analyses. Leaf rust bioassays on these clones and their progeny have also confirmed functionality of the gene. Additionally we have introduced these *Rp1-D* constructs into wheat immature embryo via biolistics and have generated transgenic wheat clones. Molecular analyses and bioassays have also been performed on these plants.

P-2005

Introduction of *cryIAc* and *bar* Genes into Kenaf (*Hibiscus cannabinus* L.). M.M. YOUNG and N.A. Reichert. Department of Plant and Soil Sciences, Box 9555, Mississippi State, MS 39762. Email: myoung@pss.msstate.edu

Kenaf's production can be adversely affected by lepidopteran insects and weeds. The introduction of a *Bacillus thuringiensis cryIAc* or a *bar* (imparts resistance to phosphinothricin-based herbicides) gene could greatly enhance cultivation. Optimized protocols were developed for DNA introduction into kenaf using *Agrobacterium tumefaciens* or the PDS-1000/He. Leaf explants of cultivars Everglades 41 and Tainung 2 were precultured on shoot regeneration media then either inoculated with agrobacterium containing plasmid pON or bombarded with plasmids pON or pLMBAR. Plasmid pON contained a synthetic, truncated *cryIAc* coding sequence driven by the 35S promoter and a *nptII* coding sequence driven by the *nos* promoter. Plasmid pLMBAR contained the *bar* coding sequence driven by the 35S promoter. Leaf explants were selected on geneticin (plasmid pON) or Ignite herbicide (plasmid pLMBAR). Several plants were recovered that contained both *nptII* and *cryIAc* genes. The *bar* gene was identified in several explants. Data on PCR analyses, Southern blots and insect bioassays will be presented.

P-2006

Functional Screening and Characterization of Tomato Genes That Block PCD Induced by TA Toxin Using the Agrobacterium rhizogenes Hairy Root Transformation System. Z.Q. PAN, X.H. Fan, R.J. Haworth, J.E. Lincoln, R.M. Bostock and D.G. Gichrist. Center for Engineering Plants for Resistance Against Pathogens (CEPRAP), University of California, Davis, One Shields Ave. Davis, CA 95616. Email: dggilchrist@ucdavis.edu

Programmed cell death (PCD) or apoptosis is a mechanism that regulates cell number and is fundamental to complex biological process of all multicellular organisms. Increasing evidence indicate that programmed cell death occurs in plants, has morphological characteristics of apoptosis, and plays an important role in disease. To identify genes that are associated with PCD in plants, we established a system for functional screening and assay of tomato PCD-related genes from a genomic library. Using this system, 5–10 Kb genomic DNA fragments from TA-toxin resistant isoline were transferred to a toxin sensitive isoline via Agrobacterium rhizogenes to generate transformed hairy roots. Transformed roots were treated with TA toxin, a toxin produced by Alternaria alternata that is a fungal pathogen that induces apoptotic death in tomato at 50 nM in untransformed or empty vector transformed sensitive roots. We screened more than 500,000 independently transformed roots with 1000 nM TA toxin and obtained 18 root clones that were resistant to three transfers in 1000 nM TA toxin. We have generated PCR products from five roots (clones C2, C3, C4, C5 and C7) using a long PCR procedure that were then cloned and sequenced. Using C7 as probe, two genomic DNA clones, C7–5G (5 Kb) and C7–10G, (8.5 Kb) were isolated by screening a tomato genomic library. Both C7–5G and C7–10G, when retransformed into the toxin senstitive background with A. rhizogenes, provided protection against TA toxin-induced PCD at 500–1000 nM. Results on isolation of cDNA clones and complementation with these PCR fragments and genomic clones will be presented.

P-2007

Differentially expressed genes in wounded stem tissue of chestnut in vitro shoots. R. SCHAFLEITNER and E. Wilhelm. Life Sciences Department, Austrian Research Centers, A-2444 Seibersdorf, Austria. Email: ROLAND.SCHAFLEITNER@ARCS.AC.AT

In vitro cultured chestnut shoots (*Castanea sativa*) were used as a model system to discover wound-responsive genes in tree species. 27 cDNA fragments of genes, induced or repressed during the first 24 hours after wounding, were isolated from stem tissue by the RT-PCR differential display method. 80% of the wound-inducible genes detected were found to be induced as early as 3 hours after wounding, the remaining 20% started to be up-regulated between 3 and 12h post treatment. The high expression of most of the wound-inducible genes was persistent for at least 24 hours. Genes induced later than 24h after wounding were not investigated in this study. cDNA fragments of genes induced after wounding showed sequence similarity to protein kinases, PR-proteins (chitinase, glucanase, pectin esterase), germins and to an oxoglutarate/malate translocator. A few repressed genes also were detected, here no significant sequence similarity to known genes could be found. A cDNA fragment with sequence similarity to an inositol polyphosphate phosphatase exhibited a complex expression pattern of its corresponding gene. The fragment was detected in untreated control plants, seemed to be repressed 3 hours after wounding and was highly induced 12 to 24 hours post treatment. However, for most of the isolated cDNA fragments no sequence similarity to previously described genes could be found. Therefore we currently screen a cDNA library established from wounded chestnut stems in order to isolate the corresponding full length clones to the isolated cDNA fragments for a further characterisation.

P-2008

Expression of a Caspase Inhibitor Protein in Tomato Plants Results in Decreased Disease. JAMES E. LINCOLN, Craig Richael, Juan Li, Kathy Smith, Xiaohong Fan, Ian Crossley, Richard M. Bostock and David G. Gilchrist. Center for Engineering Plants for Resistance Against Pathogens, University of California Davis, Davis CA 95616. Email: dggilchrist@ucdavis.edu

Programmed cell death or apoptosis, widely studied in animal systems, has been shown by our laboratory to occur in plant cells using biochemical and cell biological techniques (Wang et al. (1996) Plant Cell 8:375–391). Apoptosis is regulated by a number of genes in animal systems and results in the activation of a cascade of proteases. This family of proteases known as caspases plays a key role in the regulation of apoptosis in animal cells. Expression of the p35 baculovirus gene, blocks apoptosis in animal cells by inhibiting caspases. We have generated transgenic tomato plants which constitutively over-express p35 protein. We show these plants to be more resistant to inducers of cell death than untransformed plants. We have shown that p35 expression in tomato results in decreased disease symptoms caused by a variety of pathogens including: A. alternata f. sp. lycopercisi, the toxin producing fungus; A. alternata, the causal agent of Black Mold in tomato which does not produce any known toxins and only infects ripening fruit; Colletotrichum coccodes, another colonizer of ripe fruit for which no known natural resistance exists; Sclerotinia sclerotiorum, the causal agent of White Mold; and Pseudomonas syringae pv. tomato (Pst), a bacterial pathogen. The characterization of these p35 expressing plants with respect to plant pathogens will be presented.

P-2009

Engineering wheat for improved resistance to water deficit stress. T. ABEBE, A.C. Guenzi and B.C. Martin. Department of Plant & Soil Sciences, Oklahoma State University, Stillwater, OK 74078-6028. E-mail: abebe@okstate.edu

Water deficit stress is the single most important abiotic factor that depresses crop productivity. Efforts to improve yield through classical breeding have met with limited success primarily because resistance to water deficit stress is controlled by many genes and their simultaneous selection is difficult. Through genetic engineering resistance genes isolated from any organism can be selectively transferred to a target organism without the need for sexual reproduction. One major molecule that has been a target for engineering resistance to water deficit stress is mannitol. Mannitol protects plants through osmotic adjustment, osmoprotection of macromolecules and serves as a sink for reducing power and storage of carbon. We have transformed wheat plants (*Triticum aestivum* cv. Bobwhite) with the *mtlD* gene from *E.coli* using the biolistic technique to improve resistance to water deficit stress. *MtlD* encodes for mannitol-1-phosphate dehydrogenase which catalyses the reversible conversion of fructose-6-phosphate to mannitol-1-phosphate. The conversion of mannitol-1-phosphate to mannitol is catalysed by non-specific phosphatases. To evaluate how partitioning of mannitol between organelles influences the response of wheat to water deficit stress, two groups of plants have been created: one group expresses mannitol in the cytosol and the other in the chloroplast. Mannitol accumulation in the chloroplast has been achieved using the transit peptide sequence of Rubisco. Integration of the *mtlD* gene and its expression has been confirmed by Southern, PCR and enzyme activity assays. A total of 38 plants (with a transformation frequency of 0.5%) were recovered. Currently progress is underway to study the response of offsprings of selected plants to water deficit stress.

P-2010

Molecular and biochemical analysis of selected rice lines created by desiccation treatment of callus tissues P. T. DINH[1,2], L.T. Binh[1,2], L. T. Muoi[1,2], M. Egnin[2]. [1] Institute of Biotechnology, Hoang Quoc Viet St., Cau Giay, Hanoi, Vietnam; [2]Center for plant Biotechnology research, Tuskegee University, AL 36088, USA. megnin@tusk.edu and dinhthiphong@hotmail.com

Rice account for to 85% of the total grain production in Vietnam and is increasing annually. However, many environmental stress factors, such as drought, chilling temperature, acidified soil and saline soil, significantly reduce the productivity of this important crop and also limit the growing areas. Water stress alone affects the productions on 2.1 million ha of the total 6.58 million ha used for rice cultivation in Vietnam. Somaclonal variation has been earlier employed to select the new rice varieties with higher yield potential and drought-cold-tolerance. The selection procedure, based on desiccation treatment of in vitro cultured callus cells for dehydration resistant cell lines and for regeneration of plant lines with improved drought tolerance, has been successfully developed in Institute of Biotechnology. Three weeks old calli derived from mature seeds of the local cultivars CR203 and C70, were dehydrated by air blowing under aseptic condition until 85% of fresh weight were lost. The dried calli were recultured on callus culture medium and then on shoot regeneration medium. More than 900 green plantlets (R0) were regenerated from the survived cells, most of them were fertile (516/517). They were further tested through RAPD analysis using 11 random 10 nucleotide primers designed for Indica rice, DNA of 17 selected CR203 lines and 16 selected C70 lines showed that more than 74 different RAPD fragments have been amplified demonstrating a wide polymorphism. The dissimilarity ranged from 8% to 22 %. One line (DR2), which differs from the original cultivar CR203 by various morphological, physiological and agronomic characteristics, showed the highest dissimilarity (22%) from the CR203 in the RAPDs comparison. Analyzed data of the cold-test at the 3-leaf-seedling stage and the drought-test at the tillering stage showed that regenerated lines are superior in chilling and in drought tolerance compared to the original cultivars. Chlorophyll fluorescence measurements indicated that the photosynthetic system of selected lines were more insensitive to artificial drought by sorbitol treatment. SDS electrophoresis of seed proteins revealed modified patterns. Higher proline contents were found in selected lines compared to that of original cultivars.

P-2011

NaCl Stress Induces an Increase in Abscisic Acid Levels in Cotton Callus Tissue. D.R. GOSSETT, J. Carmody, S.W. Banks, and M.C. Lucas. Louisiana State University-Shreveport, One University Place, Shreveport, LA 71115. Email: dgossett@pilot.lsus.edu

It has been suggested that abscisic acid (ABA) plays a role in the signal transduction pathway associated with NaCl-induced upregulation of antioxidant enzymes. In this study, ABA concentrations were measured by HPLC analysis in cotton callus tissue grown at 0, 100, or 150 mM NaCl for three weeks. While the ABA levels in the callus tissue grown at 100 mM NaCl were not significantly different from the control callus, the callus tissue grown at 150 mM exhibited an approximate 75% increase in ABA concentration. Time course studies indicate that the NaCl-induced increase in ABA concentration occurs early in the response to NaCl stress. The data from this study suggest that ABA may play a role in the signal transduction mechanism associated the upregulation of antioxidant enzyme activity during NaCl stress in cotton callus tissue.

P-2012

ISOLATION AND CHARACTERIZATION OF SERINE AND PROLINE RICH PROTEIN ENCODING GENE FROM *PORTERESIA COARCTATA* T. UNDER SALT STRESS CONDITIONS. SUMEET G C BABU[1] and V D Reddy[2] [1]CNRS BP 17 Auzeville, F31326 Castanet, Tolosan, France. gondi@cict.fr [2]CPMB Osmania University Hyderabad, 500007 India.

Rice is one of the most important cereal crops in the world. It is the primary food source for 40% of the world population. Production and planting area of rice are greatly affected by soil salinity. An estimated 952 x10^6 hectares of land is affected by salinity in the world. The attempts to develop salinity tolerance in rice, through conventional breeding programmes produced no significant results due to the limited genetic variability. Many genes associated with salinity tolerance from various sources have been isolated. Most of these genes isolated belong to the following categories, namely osmolyte synthesis, ion sequestration, and water replacement protein encoding genes, besides 'LEA' group. There is a need to isolate genes responsible for membrane stabilization and water channel function associated with salinity tolerance. In the present investigation *Porteresia coarctata* T., a wild halophytic grass species has been employed as source of genes that confer salinity tolerance. Present study mainly deals with the determination of anatomical and physysiological changes associated with salinity tolerance in *Porteresia coarctata*. It was also envisaged to construct a root and rhizome specific cDNA library from salt stressed tissues besides isolation and characterization of salt inducible gene by using heterologous probe encoding a membrane stabilizing protein from *Medicago sativa*. Root and rhizome specific cDNA library was constructed in lambda gt10 from the salt stressed tissues of *Porteresia coarctata* T. Southern analysis revealed that the isolated clone is a single copy gene. Northern analysis and *in situ* mRNA expression indicated that the gene expression is confined to root and rhizome and under the control of salt inducible system. Nucleotide sequencing of the sub cloned 'PcSER' gene in pBluescript SK revealed that the gene might encode for a protein of 288 amino acids. Deduced amino acid sequence exhibited domain based homology with water channel protein, calcium channel protein besides MsPrp2. The presence of characteristic SPSPSPSS and SSSSS sequence stretches and trans membrane nature of the protein evident from the hydropathic index is indicative that the gene may encode a protein having the potential to form cell wall to membrane linkers. The gene isolated in the present investigation has been assigned an accession number AF110148 from the Gene Bank. This isolated gene may encode a unique protein involved in membrane stabilization during salt stress. Further studies are essential for the characterization of the gene product and its regulatory role in salinity tolerance.

P-2014

Comparative studies of somatic embryogenesis in *Quercus rubra* and *Juglans nigra*. M.J. BOSELA and C.H. Michler. USDA Forest Service, North Central Research Station, Hardwood Tree Improvement and Regeneration Center, 1159 Forestry Building, Purdue University, West Lafayette, IN 47907-1159. Email:mbosela@fnr.purdue.edu

Work has begun to develop somatic embryogensis protocols for northern red oak (*Quercus rubra*) and black walnut (*Juglans nigra*) for use in tree improvement programs. Somatic embryo cultures were initiated from the cotyledon explants of zygotic embryos. For each species, zygotic embryos were collected from three maternal clones at 2 to 3 week intervals from mid-July to the end of August. Nineteen types of primary media, differing in hormone content, salt formulation, or both, were employed. The hormone treatments included 2,4-D at high, medium, and low concentrations in combination with low levels of BA, and the more novel hormone combinations of earlier researchers (i.e. Tulecke and McGranahan, 1985, Aly et al., 1992, Neuman et al., 1993). Three salt formulations (WPM, MS, and DKW) were employed and hormone-free controls were included as appropriate. On each collection date, explants were harvested from 4 to 6 zygotic embryos per clone and separately maintained to assess genotype effects. The explants were transferred to secondary media after 4, 8, and 12 weeks. Three types of secondary media (supplemented with BA, 2,4-D, or hormone-free) were employed. Primary somatic embryos were excised as they formed and used to initiate embryo lines. The morphology and proliferation of embryos on hormone-free media was compared with that of embryos on auxin (IAA, 2,4-D) or cytokinin (BA, Zeatin) media. Species-specific differences will be outlined and attempts will be made to relate between-line variability to differences in genotype or primary media. The effects of different salt formulations and exogenous hormones on somatic embryo characteristics will also be discussed.

P-2016

Purine and Pyrimidine Biosynthesis During White Spruce (*Picea glauca*) Somatic Embryo Maturation. C STASOLLA[a], N. Loukanina[a], I. Ashihara[b], T.A. Thorpe[a]. [a]Plant Physiology Research Group, Department of Biological Sciences, University of Calgary, Calgary, Alberta, CANADA, T2N 1N4. [b]Department of Biology, Faculty of Science, Ochanomizu University, Tokyo, 112-8610 JAPAN. Email: cstasoll@ucalgary.ca

Pyrimidine and purine metabolism was investigated at various stages of white spruce (*Picea glauca*) somatic embryo development by following the metabolic rate of adenine, adenosine, inosine, uracil, uridine, and orotic acid. The pyrimidine de novo pathway was very active throughout embryo development, since more than 80% of [6-^{14}C] orotic acid absorbed by the tissue was utilized for neclotide and nucleic acid synthesis at all stages of development. As embryos matured, however, the decline in activity of orotate phosphoribosyltransferase paralleled the lower proportion of radioactivity from orotate recovered into the nucleic acid fraction. A large proportion of uracil was degraded as $^{14}CO_2$, probably via ß-ureidopropionate. In contrast, a significant fraction of uridine was converted into salvage products, i.e. nucleotides and nucleic acids, by the enzyme uridine Kinase (UKase), which was found to increase during maturation. For purines, inosine was extensively degraded to ureids and CO_2, whereas a fast turnover was observed for adenine and adenosine throughout the maturation period. The utilization of adenine and adenosine for nucleic acid synthesis slowly declined as embryos matured. This paralleled a decrease in specific activity of the respective salvage enzymes adenine phosphoribosyltransferase(APTRase) and adenosine kinase (AKase). Collectively, these results argue for the requirement of a large purine and pyrimidine nucleotide pool necessary to sustain cell division in developing somatic embryos. Furthermore, the increase of UKase at the end of the maturation period denotes the possibility that an active pyrimidine salvage may be necessary for the reactivation of the overall cellular metabolism at the onset of germination.

P-2018

Development of Transgenic Plants as Source of Edible Vaccine For Rinderpest Disease. ABHA KHANDELWAL, Geetha N., Vally K. J. M., Shaila M. S. and Lakshmi Sita G. Department of Microbiology and Cell Biology, Indian Institute of Science, Bangalore-560 012, INDIA. E-mail:abhak@mcbl.iisc.ernet.in

Rinderpest is a highly contagious disease of cattle, buffaloes, sheep, goats and wild ruminants with a high mortality rate. The causative agent, Rinderpest virus has only one antigenic type (serotype) and an attenuated, live vaccine with high immunogenicity is available. Rinderpest has been eradicated in developed countries, but is still prevalent in parts of Africa, the middle East and South Asia, where eradication campaigns are underway. The major drawback of the currently used Rinderpest vaccine is its heat lability. In hot countries, the vaccine delivery is constrained by high costs, lack of maintenance of cold chain to keep the potency of the vaccine. Although recombinant vaccines- vaccinia/capripox recombinant or bacculo recombinants- have been produced, they have neither been tested in the field nor their usefulness in providing long term immunity has been experimentally proven. Development of transgenic plants as source of edible vaccines delivered through the food would represent the most efficient and economical means of mass immunization of domestic ruminants as well as susceptible wild life. Towards this goal, we have undertaken to develop (a) transgenic tobacco plants (model system) and (b) transgenic peanut (*Arachis hypogea*) plants expressing the hemagglutinin (H) protein of Rinderpest virus (RPV). We present here our results in developing transgenic plants of tobacco and peanut expressing the H protein. The hemagglutinin gene of RPV from pbluescript was subcloned into the binary vector pBI 121. The recombinant binary vector pBI-H was mobilized into the hypervirulent *Agrobacterium tumefaciens* strain EHA 105. For tobacco, leaf disc method was used and for peanut embryonal axis was used as the initial explant for transformation. Presence of H gene was shown by PCR using gene specific primers. About 24 transgenic lines of tobacco and 116 transgenic lines of peanut were obtained. The integration of the transgene has been confirmed by Southern hybridization. The expression of H protein in these transgenic lines has been shown using polyclonal monospecific antibody to H.

P-2019

Transgenic Banana Containing the Hepatitis B Surface Antigen. J.M. VAN ECK, S.M. Abend, X. Sawycky, H.S. Mason, and C.J. Arntzen. The Boyce Thompson Institute for Plant Research, Tower Rd., Ithaca, NY 14853. E-mail: JV27@CORNELL.EDU

Banana (*Musa* spp.) was chosen as one of the crops for our edible vaccine program because it is the fourth most important food crop in the world and it is one of the first foods babies can eat. Suspension cultures were initiated from shoot-tip derived callus of the Indian cultivar Rasthali (AAB). These suspension cultures were then used as target material for microprojectile bombardment experiments. The suspension cultures were cobombarded with two constructs. One construct contained the nptII gene as a selectable marker and the other construct contained the coding region for the hepatitis B surface antigen (HBsAg). Two days post bombardment, cultures were transferred to medium containing kanamycin. The first putative transformants appeared 6 months after bombardment. PCR analysis of the first putatives confirmed the presence of the gene for HBsAg.

P-2020

Production of Recombinant Rotavirus VP6 from a Suspension Culture of Transgenic Tomato (*Lycopersicon esculentum* Mill.) Cells. Chang Heon Kim, Seong Hyun Hong, Jong Hwa Park, Jong Jin Lee, and IN SIK CHUNG. Dept. of Genetic Eng., Kyung Hee University, Suwon, Korea, 449-701. Email: ischung@nms.kyunghee.ac.kr

Rotavirus is a member of the reoviridae family and causes acute gastroenteritis in human infants. The major inner capsid protein of rotavirus, VP6, is associated with viral RNA polymerase activity. VP6 may bind to RNA genome segments or transcripts, or interact with other viral proteins during virus replication and assembly. Production of large quantities of VP6 polypeptide by recombinant DNA technology can be of value in evaluating the effectiveness of a subunit vaccine against rotavirus infections. In this study, we describe the expression of cDNA coding for bovine rotavirus VP6 from suspension culture of transgenic tomato cells. Recombinant rotavirus VP6 expressed in transgenic tomato cells was found primarily in the intracellular fraction with a molecular weight of 44 kDa. In a shake flask, transgenic tomato cells produced 0.35 mg recombinant VP6/L at 18 days of incubation. In a high aspect rotating-wall vessel designed by NASA to simulate microgravity, the transgenic cells produced up to 0.15 mg recombinant VP6/L. We are currently investigating the effect of sodium butyrate on cell growth and recombinant VP6 production.

P-2021

Production of Catalpol in Hairy Root Cultures of Chinese Foxglove (*Rehmannia glutinosa*). S.J. HWANG, Department of Food and Biotechnology, Dongshin University, Naju, 500-174, Korea. E-mail: sungjhwang@hanmail.net

Chinese foxglove (*Rehmannia glutinosa*) is a valuable herb medicine which has been used in traditional Chinese medicine. Hairy root cultures were used as a culture system *in vitro* for the production of medicinally important compounds to avoid many of the problems that affect the traditional production from field-grown species. We successfully established hairy root cultures of Chinese foxglove by infection with *Agrobacterium rhizogenes*. The growth and catalpol contents of hairy roots grown in flask were differently affected by basal culture media tested. The best growth of hairy roots was obtained on SH medium with 3% sucrose. The maximum production of catalpol was achieved in WPM medium containing 4% sucrose. Addition of plant growth regulators and elicitors to hairy root cultures remarkable effect on growth and catalpol contents. The transgenic plants regenerated from hairy root clones had different phenotypes. In clonal regenerants, the high capacity of rooting and catalpol contents associated with the original hairy root lines were stably maintained. For mass production of medicinal materials, hairy roots were cultured in airlift type bioreactor. The scale-up cultures did not lead to any loss in biomass yield and catalpol contents.

P-2022

Terpenoid Biosynthesis via a Non Mevalonic Acid Pathway in Transformed Roots of *Artemisia annua* L.: Cloning and Expression of DXS and DXR. F.F. SOURET[1], K.K. Wobbe[2] and P.J. Weathers[1]. Dept. of Biology/Biotechnology[1] and Dept. of Chemistry/Biochemistry[2], Worcester Polytechnic Institute, Worcester, MA 01609. E-mail:souret@wpi.edu

Artemisia annua produces a sesquiterpene lactone with an endoperoxide bridge, artemisinin that displays effective and potent anti-malarial activity. Two distinct terpenoid pathways have been found in higher plants leading to the biosynthesis of isopentenyl diphosphate, the common precursor of terpenoids. The traditional cytosolic mevalonic acid pathway originates from acetate while the newly discovered mevalonate-independent pathway initiates from pyruvate. This later pathway, postulated to be located in plastids, is known to be widespread in higher plants and two enzymes, bearing transketolase-like activity, have already been identified: 1-deoxyxylulose-5-phosphate synthase (DXS) and 1-deoxyxylulose-5-phosphate reductoisomerase (DXR). To further investigate the regulation of the sesquiterpene biosynthesis using transformed roots of *A. annua* as a plant model and to isolate genes involved in the non mevalonic acid pathway, we employed a cloning strategy, using RT-PCR, to isolate two partial cDNAs encoding for DXS and DXR. Amino acid sequence alignment showed high level of similarity among plant species (between 71 and 91%). Furthermore, a Lambda-ZAP cDNA library is being screened to obtain full-length cDNAs encoding for these two enzymes. Northern blot analysis showed that DXS and DXR were expressed not only in normal plants grown in greenhouse but also in transformed roots grown *in vitro*. Preliminary results also show that DXS is upregulated by light while DXR is not. To our knowledge, this is the first report demonstrating the presence of the non mevalonic acid pathway in transformed roots for any plant species and the first time that these genes have been isolated in *A. annua*.

P-2023

Is an ascorbate peroxidase involved in degradation of artemisinin in hairy roots? T. ISKRA[1], K.K. Wobbe[1], and P.J. Weathers[2*]. [1]Department of Chemistry and Biochemistry, [2]Department of Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01609, USA.

Peroxidases (E.C. 1.11.1.7) in hairy root cultures of *Artemisia annua* L. were characterized, and their involvement in the destruction of artemisinin (AN) was studied. For complete AN degradation, both ascorbic acid and iron (III) were required. Both degradation of AN as well as specific activity of guaiacol and ascorbate peroxidases, were affected by pH. Even under the conditions exhibiting highest degradation activity for AN, the degradation of AN was successfully prevented through the addition of hydrogen peroxide, a competitive substrate. Further analysis of *A. annua* hairy root peroxidases through isoelectric focusing revealed 9 isozymes of peroxidase with 3 of these also appearing in the culture media. All were acidic. Clarification of the role of peroxidase in the degradation of AN may suggest methods for controlling loss of AN *in vitro*.

P-2024

Synthesis and Accumulation of lipid compounds in in vitro cultures of Cynara cardunculus L.. M. J. G. VILAÇA-SILVA and M. Fernandes-Ferreira. Department of Biology, University of Minho, Largo do Paço, 4709 Braga Codex, PORTUGAL. E-mail: mfferreira@bio.uminho.pt

In vitro cultures of Cynara cardunculus were established using as primary explants nodal segments from aseptic in vitro germinated seedlings. Shoot regeneration occurred on modified MS supplemented with IBA, BA and 2ip. The substitution of BA by KIN induced the increase in the linear growth of the shoots. The transference of the shoots to the MS medium supplemented with NAA and IBA induced the rhizogenesis. Calli were obtained from different type of primary explants namely young leaves, root segments, internodal shoot segments and nodal shoot segments laid on MS medium supplemented with 2,4-D and ZEA. The extraction of lipid compounds from calli showed the presence of high contents of free constituents namely fatty acids, n-alkanols, aliphatic hydrocarbons, sterols and triterpenols. Significant variations in the contents of these lipid compound groups were found depending from the primary explant origin of the calli. Considering the calli cultures maintained on the same medium conditions, the accumulation of sterols in calli derived from root segments was 5–10 times less than in calli derived from other types of primary explants. Free sterols and free fatty acids were the major compound groups present in the lipid extracts from all type C. cardunculus calli. n-Alkanol fraction was the less represented.

P-2025

A GFP—BAR construct for switchgrass transformation. J.K. MC DANIEL, H.A. Richards, H. Sun and B.V. Conger. Department of Plant and Soil Sciences. The University of Tennessee. Knoxville, TN 37901-1071. E-mail: congerbc@utk.edu

Objectives of this research were to: (1) construct a GFP-BAR plasmid and (2) utilize this plasmid to demonstrate genetic transformation in switchgrass (*Panicum virgatum* L.). GFP and its *Act*1 promoter were excised from a pAct1IsGFP-1 plasmid with Xho1 and Sma1 restriction enzymes. The pAHC25 plasmid containing *uid*A (GUS) and *bar*, both driven by *Ubi*1, was cut with Hind III. The bar fragment, along with the pUC8 vector backbone, was isolated from the GUS fragment and ligated with Act1s-GFP to form psGFP-BAR. Embryogenic calluses were induced from young inflorescences of an 'Alamo' switchgrass genotype. These were bombarded with 1.1 micron tungsten particles coated with the psGFP-BAR plasmid. A particle inflow gun was used for delivery. Calluses were cultured on medium containing 10 mg/l bialaphos for 3–4 months. Plantlets regenerated from tolerant calluses were established in soil in the greenhouse. Leaves of 35 putative transgenic plants were rubbed with 0.1 % Basta. Two plants exhibited high tolerance to the herbicide and 50% of the pollen of these plants fluoresced bright green under blue light indicating the presence of GFP. Presence of the bar gene was confirmed by Southern blot hybridization. Supported by Lockheed Martin Energy Research Corp. under Contract No. 11X-SY 161C.

P-2026

Factors Affecting Increased Chromosomal Aberrations in Callus Cultures and Plants of Barley (*Hordeum vulgare* L.) during Transformation Process. H.W. CHOI, P.G. Lemaux and M.-J. Cho. Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA. E-mail: choihw7@nature.berkeley.edu

Compared with nontransgenic callus cultures of barley having 15% cytologically abnormal cells, a much higher percentage of karyotypic abnormality was observed in cells of transgenic callus cultures. Out of twenty-two independently transformed callus lines generated by microprojectile bombardment of immature embryos, only 7 lines appeared cytologically stable, having a relatively high percentage (35–76%) of diploid chromosomes (2n=2x=14); 5 (71%) of these lines were regenerable. Of the remaining 15 lines a high percentage (92–100%) of cells had cytological abnormalities, including ploidy changes [tetraploidy (2n=4x=28), octaploidy (2n=8x=56), and aneuploidy] and structural variations; 8 (53%) were regenerable. There was a high correlation of cytological status between callus tissues and their regenerated plants. In the current study we determined which factor(s) in the transformation process were responsible for the increased cytological aberrations. Only 18% of 6- and 12-week-old callus cells had observable cytological changes in the absence of transformation. In contrast, imposing selection conditions and/or osmotic treatment appeared to trigger extensive cytological aberrations, 40% and 38% in 6-week-old callus cultures and 48% and 52% in 12-week-old callus cultures, respectively. However, bombardment itself did not appear to affect the frequency of cytological aberrations in cells of callus cultures.

P-2027

Quantitation of Transgenes in Soybean (Glycine maxL.) by Real Time PCR. M.A. SCHMIDT and W.A. Parrott. Department of Crop and Soil Sciences, University of Georgia, Athens, GA 30602.

A quantitative real-time PCR assay was used to determine the zygosity of transgenes in soybean. TaqMan™ assay allows direct detection of a PCR amplification product. The technology is based on a fluorescently labeled DNA probe that hybridizes to a PCR target sequence flanked by the PCR primers. By utilizing the nuclease activity of Taq polymerase, with each polymerization reaction a single fluorescent molecule is liberated. The amount of fluorescence produced during thermocycling is directly related to the starting number of DNA copies of the gene in question. That is, the fewer the cycles necessary to reach a certain level of fluorescence, the greater the number of target sequences present in the template. By performing TaqMan™ assays on increasing amounts of a plasmid containing the transgene of interest, a linear relationship of the level of fluorescence to the template from 0.2 to 100 pg., equivalent to 1 to 500 copies of the soybean genome, respectively, was produced. By using this linear relationship as a standard curve, the zygosity of transgenic lines was determined. In this manner, TaqMan™ assays were performed in triplicate using DNA from Bt-transgenic soybean lines. A zygosity assay, sufficiently sensitive to differentiate between homozygote and hemizygote transgenic lines, was designed. This zygosity data was confirmed by conventional methods, namely, Southern analysis and progeny segregation.

P-2028

Transgenic Tropical Maize with the cry1E Gene Presents Resistance to Fall Armyworm (*Spodoptera frugiperda* J.E.Smith). N. BOHOROVA[1], Royer M.[2], R. Frutos[1], P. Estañol[1], M. Pacheco [1], Q. Rancón-Cruz [1], S. McLean [1], and D. Hoisington [1]. [1] CIMMYT Int., Apdo. Postal 6-641, 06600 Mexico, D. F., Mexico; [2] CIRAD, BP 5035, 34032 Montpellier Cedex 1, France. E-mail: n.bohorova@cgiar.org;

An alternative strategy for crop protection against insect damages involves transgenic plants that express insecticidal *Bacillus thuringiensis* d-endotoxin genes. The *cry*1E gene produces a d-endotoxin crystal protein that, according to bioassays, is active against *Spodoptera frugiperda* and *Sesamia calamistis*, one of the major African corn borer pests. The synthetic *cry*1E gene was produced for better expression of the proteins in plants active for fall armyworm. The synthetic *cry*1E gene was cloned into a plant expression vector under the control of a maize ubiquitin or a rice actin-1 promoter, and linked in the single plasmid with a selectable *bar* gene under the control of cauliflower mosaic virus (CaMV) 35S promoter. The scutelum part of immature embryos from CIMMYT inbred lines and hybrids was used as the target for transformation. Transgenic plants have been selected by screening for resistance to the herbicide Basta ™ in a biosafety greenhouse. Molecular analyses confirmed the integration, copy number, and transmission of the introduced *cry*1E gene. Insect feeding bioassays indicate that 12% of the transgenic plants contained high levels of gene expression and resistance to *Spodoptera frugiperda*.

P-2029

Endosperm-specific GFP Expression Driven by Barley D-Hordein Promoter and Its Inheritance in Transgenic Barley and Wheat Plants. M.-J. Cho, H.-K. KIM, H.W. Choi, B.B. Buchanan and P.G. Lemaux. Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA. E-mail: mjcho@nature.berkeley.edu

Barley (*Hordeum vulgare* L.) hordeins are major seed storage proteins that accumulate in protein bodies of the developing starch endosperm. D-hordein accounts for about 2 to 5% of the total hordein fraction and is encoded by a single gene. In order to test the functionality of the D-hordein 5' regulatory region, the expression and inheritance of green fluorescent protein (GFP) were determined in barley and wheat (*Triticum aestivum* L.) plants transformed with a synthetic green fluorescent protein [*sgfp*(S65T)] gene driven by barley endosperm-specific D-hordein promoter. Strong GFP expression driven by the barley D-hordein promoter was found specifically in the endosperm tissue of developing barley and wheat seed; GFP expression was not observed in immature embryo tissues. Endosperm-specific GFP expression in both transgenic barley and wheat plants was stably inherited in T_2 and later generations. We conclude that D-hordein promoter can be used to develop a system for limiting foreign gene expression exclusively to the endosperm of barley and wheat seed.

P-2030

GFP to Determine Transformation Patterns in Cotton. R.H. SMITH, S.H. Park and M.G. Salas. Department of Soil & Crop Sciences, Texas A&M University, College Station, TX 77843. Email: rsmith@tamu.edu

Transformation of the shoot apex explant using *Agrobacterium* is a genotype independent transformation system. However, the frequency of transformation is low and chimerial transformation can occur. In order to improve transformation frequencies and determine which cells of the shoot apex are targeted, a visual marker of transformation would be useful. The effects of the addition of a supervirulent plasmid was tested in cotton shoot apex transformation experiments. The green fluorescent protein (GFP) was used as an indicator to visualize the target cells that expressed the GFP. *Agrobacterium tumefaciens* LBA4404 with and without an additional copy of a supervirulent plasmid with *vir*G and *vir*E genes was tested to determine the effect on transformation. The GFP expression in the explants was significantly enhanced when *vir*G and *vir*E genes were included. The number of fluorescence spots per explant was increased as well as the total number of explants expressing GFP.

P-2031

Sonication-Assisted *Agrobacterium*-Mediated Transformation of Embryogenic Soybean Tissue and Subsequent Monitoring of Gene Expression with GFP. K.M LARKIN and J.J. Finer. Department of Horticulture and Crop Science, The Ohio State University, Wooster, OH 44691. Email: larkin.23@osu.edu

Agrobacterium-mediated transformation is a widely used method for introducing foreign DNA into a broad array of plants. Although a dicotyledonous plant and a host for *Agrobacterium*, soybean has proven difficult for *Agrobacterium*-mediated transformation. SAAT or Sonication-assisted *Agrobacterium*-mediated transformation is a new transformation technique, which has been used for DNA introduction into various tissue types and plant species. SAAT utilizes sonication of plant tissue in the presence of *Agrobacterium*, which results in the production of microwounds on the surface and deep within the plant tissue, creating an entry point for the bacterium. In this study, SAAT was evaluated using embryogenic tissue of soybean maintained on semi-solid media. Successful transient and stable transformation of SAAT-treated soybean tissue was monitored using a plant expressible, ER-targeted, green fluorescent protein (GFP) from jellyfish (*Aequorea victoria*). The factors which most strongly influenced transformation of SAAT-treated embryogenic soybean tissue were the quality of the target tissue, sonication duration, and subculturing of the tissue 2 weeks following SAAT. The presence of the GFP protein was initially observed in transiently expressing cells 4 days following inoculation. Gfp gene expression declined rapidly thereafter and was only observed in selected clones, obtained 3 months after SAAT treatment. In newly generated clones, GFP expression could not be followed from a single transformed cell but seemed to arise only after a critical mass of tissue was obtained. The use of embryogenic soybean tissue maintained on semi-solid media, together with monitoring gfp gene expression may lead us to a more efficient transformation method for soybean and also increase our understanding of gene expression and regulation.

P-2032

Hypocotyl-Based Generation of Transgenic Soybean and Progeny Analyses. L. CHEN[1], J. M. Tyler[2] and N. A. Reichert[1]. [1] Department of Plant and Soil Sciences, Box 9555, Mississippi State, MS 39762. [2] Delta and Pine Land Company, P.O. Box 157, Scott, MS 38772. E-mail: Lchen@ra.msstate.edu

A hypocotyl-based organogenic regeneration protocol for soybean was coupled to biolistics-based transformation using PDS-1000/He. Plasmid pLMBAR, which contained the *bar* coding sequence controlled by the CaMV 35S promoter was used in double bombardments with gold. Post-bombardment, tissues were placed on selective shoot initiation and elongation media containing Ignite (phosphinothricin-based herbicide). Stable integration of *bar* was confirmed via polymerase chain reaction (PCR) analyses, with positive shoots rooted, transplanted to soil, acclimatized and maintained in the greenhouse. T_0, T_1, and T_2 plants of genotype PI 398469 and T_0 plants of Hill all displayed normal morphologies and were fully fertile. Southern analyses will be conducted to determine numbers of copies and integration sites.

P-2033

Generation of Transgenic Creeping Bentgrass (*Agrostis palustris* Huds.) Plants from Mature Seed-derived Highly Regenerative Tissues. M.-J. CHO, K.V. Le, D. Okamoto and P.G. Lemaux. Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA. E-mail: mjcho@nature.berkeley.edu

Mature seeds of creeping bentgrass (*Agrostis palustris* Huds. cv. Putter) was placed on medium containing 2,4-dichlorophenoxyacetic acid (2,4-D) (4.5 or 9.0 μM), 6-benzylaminopurine (BAP) (0, 0.44 or 2.2 μM) and cupric sulfate (0.1 or 5.0 μM) under dim-light conditions to induce and proliferate highly regenerative tissues. Highly regenerative tissues were transformed with a mixture of three plasmids containing the genes for hygromycin phosphotransferase (*hpt*), phosphinothricin acetyltransferase (*bar*) and ß-glucuronidase (*uidA*; *gus*) at a molar ratio of 1:1:1. Of 296 individual explants bombarded, fifteen independent transgenic lines (5.1%) were obtained after an 8- to 16-week selection period for hygromycin resistance with 30 to 100 mg/L of hygromycin B; regenerability of transgenic lines was 93%. Presence and stable integration of transgene(s) in transgenic plants were confirmed by PCR and DNA blot hybridization. Coexpression frequency of all three transgenes (*hpt/bar/uidA*) in transgenic creeping bentgrass plants was 27%; for two transgenes (*hpt/bar* or *hpt/uidA*) coexpression frequency was 53–57%.

P-2034

Transgenic Plants of Kentucky Bluegrass (*Poa pratensis* L.) Generated from Mature Seed-derived Highly Regenerative Tissues. C.D. Ha, P.G. Lemaux and M.-J. CHO. Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA. E-mail: mjcho@nature.berkeley.edu

An efficient method to produce highly regenerative tissues from a previously recalcitrant cultivar of Kentucky bluegrass (*Poa pratensis* L. cv. Kenblue) was established using medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D; 4.5 or 9.0 μM), 6-benzylaminopurine (BAP; 0.44 or 2.2 μM), and a high level of cupric sulfate (5.0 μM) under dim-light conditions (10 to 30 μE, 16 h-light). The tissues were transformed with three plasmids containing the genes for hygromycin phosphotransferase (*hpt*), ß-glucuronidase (*uidA*; *gus*), and a synthetic green fluorescent protein gene [*sgfp*(S65T)]. From 507 individual explants bombarded, ten independent transgenic events (2.0%) were obtained after a 3- to 4-month selection period for hygromycin resistance using 30 to 100 mg/L hygromycin B; of the ten independent events, seven (70%) were regenerable. Stable integration of transgene(s) in transgenic plants was confirmed by PCR and DNA blot hybridization. Coexpression frequency of all three transgenes was 20%; for two transgenes, either *hpt/uidA* or *hpt/sgfp*(S65T), coexpression frequency was 30–40%.

P-2035

Identification of a Novel Constitutive Maize Promoter and Characterization of Its Expression in Transgenic Maize. S.M. JAYNE, D. Liu, K. Hagemann, M. Mitchell, and D. Rice. Pioneer Hi-Bred International, Inc., Johnston, IA 50131. E-mail: jaynesm@phibred.com

One of the most important components in development of transgenic traits is a promoter which provides reliable, high level expression of the introduced transgene in the target cells. In plant transformation research, high levels of expression of the selectable marker gene should facilitate efficient selection of transformants. The maize ubiquitin promoter is frequently used as a constitutive promoter in maize transformation. However,use of alternative promoters is desirable when stacking multiple genes in transgenic plants, and may be used to avoid gene silencing phenomena often thought to be due to the use of homologous promoter sequences present in a construct. We have identified a novel nucleotide sequence for a promoter isolated from a maize gene encoding histone H2B, and will describe results from transformation experiments that characterize its strong, constitutive expression using both the gus reporter gene and the pat selectable marker gene.

P-2036

High Frequency Shoot Regeneration from Immature Embryo Culture in Sorghum. T. HAGIO and Y. Ohkawa. Laboratory of Cell Engineering, National Institute of Agrobiological Resources, 2-1-2 Kannondai, Tsukuba, Ibaraki, 305-8602 JAPAN Email:hagio94@abr.affrc.go.jp

An efficient shoot regeneration from immature embryo culture in sorghum was developed. Eleven genotypes were examined for potential use in tissue culture, and optimization of the tissue culture system was attempted. The tissue cultures were initiated from immature embryos taken approximately two weeks after flowering. Plants were stably regenerated from tissue culture derived from immature embryos when appropriate genotypes were used. 'C. Kafir' and 'PE932 025' showed the highest frequency of callus induction and regenerable callus formation under appropriate culture conditions. It appeared that the response in tissue culture of sorghum immature embryos varies with genotype and that cytokinins (kinetin or BA) played an important role in callus induction stages. Among the plant hormones tested, the combination of auxin (2,4-D) and cytokinin (kinetin or BA) in callus induction stages was found to be important for inducing regenerable calli. In plant regeneration stages, shoot formation was stimulated on a hormone-free medium, but the formation was inhibited on the media containing higher concentrations of cytokinins. In many cases, shoot formed through morphogenesis of adventitious buds and distinct somatic embryogenesis was not observed. The addition of proline and PVP also proved to be effective for enhancing shoot formation. For example, the frequency of shoot formation reached 100% in 'PE932 025'. These techniques may be useful for transformation studies in sorghum. Fertile transgenic sorghum plants (hygromycin or geneticin resistant) were obtained by particle bombardment using this culture system.

P-2037

Transient Gene Expresson Studies in Juncus Accuminatus (Bull Rush). S.D.ROGERS and K.S.Sarma. Bioscience Department, Salem-Teikyo University, Salem, WV 24626. E.mail: ROGERS@SALEM-TEIKYO.WVNET.EDU

Calli were initiated from in vitro grown seedlings of Juncus accuminatus cultured on Murashige and Skoog(MS) medium supplemented with picloram. Calli were infected with *Agrobacterium tumefaciens* containing the *gus* gene driven by different promoters. Among the promoters tested the super promoter gave the highest frequency (60–70%) of transient gene expression, as measured by histochemical staining. The effects of different promoters, bacterial density, cocultivation period, culture environment and calli age on transient gene expression will be discussed.

P-2038

Production of transgenic wheat at CIMMYT: One year of data. McLean S., A. PELLEGRINESCHI, L. Velazquez, R. Hernandez, R.M. Brito, and D. Hoisington. Applied Biotechnology Center, CIMMYT. Email A.Pellegrineschi@cgiar.org

This work describes the experience of the International Maize and Wheat Improvement Center (CIMMYT) in the production and handling of mass production of transgenic plants. The first was to standardize the procedure and keep the production of the transgenic plants constant. The transformation technique involves subjecting the plant tissue to microprojectile bombardment with high-quality plasmid DNA together with reduced levels of tissue culture manipulation. The frequency of transformation obtained was variable over the course of the year, with a range of 0.00 to 18.00%. Once the laboratory procedure was standardized, it became evident that the physiological status and health of the donor plants must be maintained at consistent level. Data about sun intensity, humidity, temperature, and photoperiod were analyzed and compared with the transformation efficiency. Reduction of light intensity and stresses to the mother plants were the most influential factors related to the transformation efficiency. Relationships between these factors are being further investigated.

P-2039

Genetic Transformation of Elite Oat Cultivars. A.R. CARLSON and H.F. Kaeppler. Dept. of Agronomy, University of Wisconsin, 1575 Linden Drive, Madison, WI 53706. Email: arcarlson@students.wisc.edu

The majority of cereal transformation studies utilize cultivars that are amenable to tissue culture, but are agronomically inferior to elite field cultivars. Low transformation and regeneration efficiencies have forced scientists to ignore elite cultivars in favor of those that can produce the maximum number of transgenic lines. Transformation of elite lines allows transgene phenotype evaluation in an optimal background and reduces or eliminates the multiple backcrossing generations required to introgress the transgene into commercial cultivars. This study used microparticle bombardment to deliver transgenes, and gfp-based visual screening to select transgenic, embryogenic oat callus. The transformation efficiency of elite cultivars Belle, Vista and Gem as well as elite test lines X6984-3 and X7066-5 were compared to the Gaf/Park experimental oat line that has been used in most oat transformation studies to date. We compared Belle, Vista and Gaf/Park in a study that was replicated seven times with two plates of each genotype per replication. We also compared X6984-3 and Gaf/Park twice with three plates of each genotype per replication. An additional thirteen plates of X6984-3, five plates of Gem, one plate of X7066-5 and three plates of Gaf/Park were bombarded in an attempt to regenerate transgenic elites. The Gaf/Park line produced more than double the visually-screened fluorescent callus of any line tested, and exhibited the highest transgenic plant regeneration efficiency. Results of molecular analyses and estimates of transformation efficiencies for elite versus Gaf/Park transformation will be presented.

P-2040

Somatic Embryogenesis and Genetic Transformation of South African Sweetpotato Cultivars. C. L. DANIELS*, M. Egnin and C.S. Prakash. Center for Plant Biotechnology, Tuskegee University, Tuskegee, AL 36088. Email: chantald1@hotmail.com

Although sweetpotato [*Ipomoea batatas* L. (Lam.)] improvement via genetic engineering is well documented, these improvements have been restricted to only a few elite commercial varieties due to a lack of reliable and reproducible regeneration systems. Recently, an efficient *in vitro* plant regeneration system, characterized by the continuous production of somatic embryos from leaf explants, was developed for a diverse range of sweetpotato genotypes by Egnin *et al* (Personal communication). This regeneration protocol, involving a two-phase callus production stage of three weeks in the dark followed by an embryo production stage in the light, was used to screen and test the regeneration efficiency of several South African (SA) sweetpotato cultivars. An optimal embryogenic response was obtained in only five of the SA sweetpotato cultivars (Ribbok, Blesbok, Mafutha, 84-2-20 and 94-8-1) tested and the somatic embryos obtained readily germinated into normal plantlets when cultured on basal multiplication media. *Agrobacterium*-mediated transformation from leaf explants of the five SA cultivars identified earlier were performed with strains C58 and EHA101, each harboring the binary plasmid, pIG121Hm, with an intron containing a ß-glucuronidase gene (*Uid A*). Gus histochemical analysis was used to determine the transformation efficiency of cocultivated explants, which ranged from 10% to 90%. All the cultivars were more amenable to transformation with the *Agrobacterial* strain EHA101 than C58. These cocultivated explants have been transferred to various selective regeneration media for further development into embryos. Putative transformants will be tested for the presence of the *Uid A*-intron by PCR and Southern analysis. Research supported by USDA-ARS and NASA.

P-2041

Transformation of synthetic protein gene into Vietnamese sweetpotato cultivars by *Agrobacterium tumefaciens* D. T. PHONG[1,2], P. B. Ngoc[1,2], M. Egnin[2], C.S. Prakash[2], L.T. Binh[1]. 1Institute of Biotechnology, Hanoi, Vietnam; 2Center for Plant Biotechnology Research, Tuskegee University, AL 36088, USA. (megnin@tusk.edu; prakash@tuk.edu. dinhthiphong@hotmail.com)

Sweetpotato *(Ipomoea batatas L.)* is a staple food crop and a major source of dietary protein in Vietnam (VN) especially for those farmers with limited resources. To improve the nutritive value of this crop, a synthetic gene *(asp-1)* coding for a storage protein rich in essential amino acids and also known to increase the total protein content, was introduced into cultivars K51, HoangLong, TrangTuNhien, and ChiemDau using *Agrobacterium tumefaciesn* . Preliminary studies aimed at optimizing the transformation efficiency were conducted using gusA-intron (GI) gene. Leaf and petiole explants were immersed in the suspension of *Agrobacterium* strains EHA101 and C58 containing GI for 10–20 min and cocultivated for 5–7 days the callus production media containing the 2.4-D and immediately subjected to Gus histochemical assay. The mean percent area of dark blue zone ranged in leaf explants from 20.15 % to 27.8% and from 15% to 21.7% with C58/GI and EHE101/GI, respectively, and in petiole explants from 23 % to 43 % and from 17 % to 48 % with C58/GI and EHA/GI, respectively. The *Agrobacterium* strain C58 was superior in facilitating the transfer of gusA gene to VN'sweetpotato cultivars compared to the strain EHA101. Transformation efficiency of petioles was higher than leaves for most of VN'sweetpotato cultivars. The optimized transformation protocol was used to introduce the CaMV-35S driven– 296 bp gene *(asp-1)* in VN'sweetpotato cultivars. Explants were cocultivated and selected on callus production media containing different concentrations of 2.4-D and Tiba, respectively. Developed calli were selected on kanamycin until embryos production on the embryogenic media. The germinated plantlets are transferred to a multiplication medium for further development. Recovered transformants are being tested for the *asp-1*gene integration and expression using PCR, Southern, Western and protein analyses. This project is supported by a grant of ISAAA.

P-2043

Somatic Embryogenesis in *Limonium bellidifolium*(Gouan) Durmort. Plumbaginaceae. M.A.M.ALY and Bala Rathinasabapathi. Horticultural Sciences Department, P.O. Box110690, University of Florida, Gainesville, FL 32611-0690. E-mail: mohammed_aly@hotmail.com and brath@gnv.ifas.ufl.edu

Many members of the genus Limonium, (Plumbaginaceae), are economically important ornamental species. *In vitro* plant regeneration has been reported for a limited number of species of this family. However, somatic embryogenesis has not been described. This study aims to develop and optimize plant regeneration protocols via somatic embryogenesis to propagate commercially important *Limonium* cultivars. Hypocotyl and cotyledon explants from perennial statice *L. bellidifolium*(*Statice caspia* Willd.) were cultured on Murashige and Skoog's medium supplemented with 1 $mg.l^{-1}$2,4-D at 26°C under 16h light period (PPFD 46 $micromol.quanta.m^{-2}.s^{-1}$). Embryogenic calli developed over a period of 10 to 15 days. Somatic embryos developed from calli of both explants and germinated into seedlings. Explants from *Limonium sinuatum, L. latifolium, L. aureum* and *Armeria maritima* responded variably to the same physiological parameters provided *in vitro*, suggesting a G x E influence on somatic embryogenesis in this family.

P-2044

Development of somatic embryos of *Coffea arabica*: from one cell to cotyledonary stage. Quiroz-Figueroa F. R., R. Rojas-Herrera and V. M. LOYOLA-VARGAS. Experimental Biology Unit, Centro de Investigación Científica de Yucatán, Apdo. Postal 87, Cordemex, Yucatán, México.fquiroz@cicy.mx; vmloyola@cicy.mx

Somatic embryogenesis is the processes by which somatic cells develop into plants trough characteristic embryological stages without fission of gametes. Somatic embryos (embryoid) are morphologically and physiologically similar to zygotic embryos. Somatic embryogenesis is an ideal system to investigate differentiation process in plants. However, there is not an histological study that showing their development from one single cell to the final stages through the different stages of development. In this work we present the ontogenesis of embryoid of *C. arabica* cv. Caturra Rojo induced into liquid medium. The early division until embryoid of 16 cells is very similar to early division in animals (morula stage). Six general stages of development of the embryos were observed: 1) globular, 2) elongated, 3) heart, 4) early torpedo, 5) torpedo and 6) cotyledonary. Four substages were observed in the globular stage; we also observed the formation of cambium since the globular stage. In this work we demonstrated that the sequential events in the embryo development are conserved and arise through one unicellular pathway. This work was supported by CONACYT; grant No. 4123P-N

P-2045

In Vitro Regeneration of Cowpea by Thidiazuron. L.E. MARSH. Cooperative Research, Lincoln University, Jefferson City, MO 65101. Email: marshl@lincolnu.edu

Seedling explants of cowpea (Vigna unguiculata) were tested for their regeneration potential in ten thidiazuron (TDZ) levels ranging from 0 to 40 micromoles. Seeds were germinated for 3–4 days on Gamborg's B-5 basal medium containing 1% agar. Cotyledons, cotyledonary nodes, hypocotyls, epicotyls, primary leaves and apical sections were cultured in media containing Murashige and Skoog Basal Salt, 3% glucose, 0.2% phytagel, myoinositol, thiamine and TDZ. Shoots were produced from nodes, apical sections and the proximal ends of cotyledons within one to three weeks of TDZ treatment. Following subcultures on hormone free media, multiple shoots developed and produced roots. High TDZ levels delayed shoot growth.

P-2046

Patterns of Variation of *n*-alkanes During In Vitro Induction of Somatic Embryogenesis from Flax Hypocotyls (*Linum usitatissimum* L.) A.C. CUNHA and M. Fernandes-Ferreira. Department of Biology, University of Minho, Campus de Gualtar, 4710 Braga, PORTUGAL. Email: accunha@bio.uminho.pt

The ubiquitous presence of normal alkanes as constituents of leaf cuticular waxes is well established and both their biogenesis and their value as a taxonomic criterion have been extensively discussed. Variations in *n*-alkanes depending on plant species and cultivars, the organ type, the leaf age, the carbon metabolism in cell suspensions and the plant adaptation to stress conditions have been reported. Lipid biosynthesis is developmentally regulated in plants, but, although the use of *n*-alkanes as a chemotaxonomic aid is very common, the use of these lipids as a trend marker aid to study in vitro developmental process has not been described. Recently, differences between the lipid biosynthesis pathway of embryogenic tissues when compared to non-embryogenic calli were reported. However, little is known about lipid modifications that occur during the onset of somatic embryogenesis. Hypocotyl segments of flax seedlings, germinated in vitro, were used to induce indirect somatic embryogenesis on solid medium. GC and GC-MS have been used to study the composition and distribution of *n*-alkanes in tissues harvested at different developmental stages. In all tissue samples a gaussian-like distribution of low M, *n*-alkanes (C13-C21) was found. The highest proportions of *n*-alkanes occurred in the explants and in the early stages of calli differentiation, with no distinction between even or odd carbon number alkanes. During the expression of somatic embryogenesis important quantitative and qualitative changes in the *n*-alkane profile were observed in embryogenic, non-embryogenic and somatic embryos.

P-2048

Regeneration of F1 Hybrids Derived From Crosses Between Cultivated Alfalfa and a Highly Regenerable Regen SY Line. J. WILL and S. Austin-Phillips. University of Wisconsin Biotechnology Center, 425 Henry Mall, Madison WI 53706. Email: Jessie_Will@gene.biotech.wisc.edu

Cultivated alfalfa (Medicago sativa) is an example of a crop species that has few breeding lines easily amenable to regeneration or genetic transformation. Genotypes have been developed which are amenable in tissue culture, the so-called Regen lines, (Bingham, 1975) and the trait for somatic embryogenesis is known to be heritable. F1 seeds obtained from crossing non-regenerative breeding lines of Legendairy (L) and Blazer XL (BL) to a highly regenerative clone, RSY#27 (Bingham, 1991) were screened for somatic embryogenesis using a modified method as described by Chen, 1987. Seeds were germinated *in vitro* and individuals tested for their ability to regenerate on several different media regimes. The frequencies of regeneration through somatic embryogenesis in the hybrid population ranged from 40–75%. Currently, tests are underway to determine if one of these clones can be transformed using *Agrobacterium*. These results clearly demonstrate the feasibility of combining cultivated and Regen germplasms to widen the genetic base available for use in alfalfa transformation

P-2049

Shoot Organogenesis from Nodal Explants of Corn. M.M. YOUNG and N.A. Reichert. Department of Plant and Soil Sciences, Box 9555, Mississippi State, MS 39762. Email: myoung@pss.msstate.edu

Optimized protocols were developed for adventitious shoot production from nodal sections excised from corn seedlings. Seeds were surface sterilized on media + BA and nodal section explants were excised from 4–7 day-old seedlings. Shoot initiation was observed as early as two weeks after culture. Histological analyses of explants excised from 7 day-old seedlings determined that, initially, the explant did not contain the shoot tip or axillary buds. Adventitious shoots appeared to arise from parenchyma cells on these explants. Shoots were elongated, rooted and acclimatized. Plants were placed under greenhouse conditions where normal flowering and seed set occurred. Data will be presented on percentage shoot response and number of shoots/explant for several cultivars.

P-2051

Regeneration from Nine Maturity Groups of Soybean via Hypocotyl-Based Organogenic Regeneration. A.L. WOODS, L. Chen and N.A. Reichert. Department of Plant and Soil Sciences, Box 9555, Mississippi State, MS 39762. Email: awoods@pss.msstate.edu

Hypocotyl explants were excised from seedlings representing 18 genotypes (public lines) and 9 maturity groups (MG 0–8) of soybean following the procedure of Dan and Reichert (IVCDB 34P:14–21, 1998) developed for MG 4–6. Genotypes were kindly provided by J.M Tyler (Delta and Pine Land, Scott, MS) and J.R. Wilcox (USDA-ARS, West Lafayette, IN). Adventitious shoots were generated on explants from all genotypes tested, although genotype differences in explant response and number of shoots generated per explant were noted. These differences could not be attributed to differences in MG, although only two genotypes per MG were tested. This research confirms the genotype-independent and MG-independent nature of the hypocotyl-based organogenic regeneration procedure developed for soybean. As such, this procedure may be applicable to numerous other soybean genotypes of interest.

P-2052

Effect of Different Auxin and Sugar Treatments on Callus Induction, Embryogenesis and Plantlet Regeneration from Mature Embryos of Wheat (*Triticum aestivum* L.). M.G. MENDOZA and H.F.Kaeppler. Dept. of Agronomy, Univ. of Wisconsin, Madison, WI 53706. E-mail: mgmendoza@facstaff.wisc.edu

Genetic engineering of plants depends upon a reliable plant regeneration system. In wheat (*Triticum aestivum* L.), immature embryos are the most widely used explant source, but they are inconvenient due to their temporal availability and maintenance requirements. Mature embryos are easily stored and available at all times in the form of mature seeds. However, plant regeneration frequencies from cultures derived from mature embryos are generally much lower compared to those initiated from immature embryos. Organic additives, and particularly the type, concentration, and balance of growth regulators are crucial in determining tissue culture responses. Additionally, the type of carbon source seems to influence the callus growth and plant production of cereal *in vitro* cultures. Replacement of sucrose by other sugars such as maltose has been found to significantly increase plant regeneration frequencies in cereal tissue cultures. However, the effect of substituting sucrose by maltose on cultures derived from wheat mature embryos has not been reported. This research was undertaken to compare the effect of four auxins (2,4-D, Dicamba, Picloram, and 2-MCPP) in three different concentrations, and to evaluate the effect of maltose versus sucrose under filter sterilized and autoclaved conditions, on callus induction, embryogenesis, and plant regeneration responses from wheat mature embryos of cultivar 'Bob White'. The experimental unit consisted of one petri-plate containing 6 dissected embryos cultured on Murashige and Skoog (MS) basal media and vitamins supplemented with the respective hormone and sugar treatments. The full experiment was replicated 6 times. All auxin treatments resulted in callus induction except 2-MCPP. Presence of Picloram in the culture media significantly enhanced callus growth, though with low embryogenic and plant regenerability. Use of Dicamba resulted in significantly higher number of plantlets. A highly significant effect of auxin type on callus and plantlet production was detected, though interactions were observed, and will be discussed.

P-2053

Somatic Embryogenesis from Nucellar Tissue of Cashew (*Anacardium occidentale* L.). VINITHA CARDOZA and L. D'Souza. Laboratory of Applied Biology, St Aloysius College, Mangalore 575003, India. Email: vinu_cardoza@hotmail.com

Cashew is an important nut crop with an annual world trade of more than 600 million US$. India is one of the largest exporter of cashew nuts. The current production of nuts is insufficient to meet the demand of the international market due to dearth of elite planting material. To supplement the available planting material at present, there is an urgent need for *in vitro* propagation of cashew. In vitro propagules have been obtained from juvenile tissues. There are no reports of morphogenesis or embryos from tissues of mature trees. This communication reports the induction of somatic embryos from nucellar tissue of cashew. Immature nuts were used for the experiments. The pericarp of the nut was cut open and the seed was dissected out. It was cut vertically into two halves and cultured on MS medium with 20g/l sugar, 0.1g/l m-inositol and 0.5 mg/l picloram after discarding the zygotic embryo, with the nucellar tissue touching the surface of the medium. Embryogenic callus and proembryos were formed from the nucellus. Globular embryos were formed on subculturing the proembryos on MS medium with 0.5 mg/l picloram and 1 mg/l putrescene. For maturation, the globular embryos were cultured on MS medium with 30 g/l sucrose and 0.5 mg/l ABA. The mature embryos germinated when transferred to MS medium with 20 g/l sucrose, devoid of growth regulators. This work opens up frontiers for micropropagation of cashew to produce true to type plants from adult trees.

P-2054

Investigation of the interspecific hybrid Capsicum baccatum var. pendulum (Wild.) Eshbaugh x C. annuum L. grown via embryoculture technique. R. PANDEVA, V. Nikova, R. Vladova. Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria. E-mail:r.pandeva@netcourrier.com; v.nikova@bas.bg

Crosses between Capsicum baccatum var. pendulum and C. annuum are difficult for realization because of the existent barriers of incompatibility. When C. annuum (cv. Byala kapiya) was used as pollinator, small amount of underdeveloped seeds was obtained. They were cultured on Murashige and Skoog agar medium for embryos, supplemented with 0,05 mg/L of gibberellic acid, kinetin and (-naphthaleneacetic acid. F1 plants obtained were with intermediate habit, well-developed flowers with yellow spots at the base of corolla lobes (typical of C. baccatum), yellow anthers with purple lateral band and 12,89% pollen fertility. Three types of flowers were observed after one backcross with C. annuum: identical with these of F1 plants but with reduced to 8,06% pollen stainability; with rudimented band-shaped anthers without sporogenic tissue and antherless ones. In the process of recurrent crossing (3–4 BCís) the plants acquired C. annuum habit but maintained their sterility. Most of them were antherless or with rudimentary stamens. In rare cases plants with purplish blue, well-developed minute anthers with no viable pollen appeared. Comparative analysis of several enzymes in leaves of parents and BC plants were performed. Obviously the observed sterility is caused by the interaction of C.annuum genes and C. baccatum var. pendulum cytoplasm.

P-2055

Cell Suspension Culture of *Persea pachypoda* and *P. cinerascens*. WITJAKSONO and Richard E. Litz. Tropical Research and Education Center, University of Florida, 18905 SW 280 St., Homestead FL 33031-3314. E-mail: Witjak@gnv.ifas.ufl.edu.

Suspension cultures of *Persea pachypoda* and *P. cinerascens* were initiated from 1 cm nodal explants from *in vitro* seedlings in 40 ml liquid plant growth medium in 125 ml Erlenmeyer flasks. The medium contained modified MS salts with molar NH_4NO_3/KNO_3 ratio of 1:2, 170 mg l^{-1} NaH_2PO_4, 100 mg l^{-1} myo-inositol, 4 mg l^{-1} thiamine HCl, 5 mg l^{-1} BA, 0.5 mg l^{-1} NAA and 30 g l^{-1} glucose. Cells were released after 21 days of culture for *P. pachypoda*, and after 2 months for *P. cinerascens* when the explants had turned black. The cell suspensions consisted of a mixture of necrotic and healthy cells, of which the latter increased with subculture. The suspension cultures were transferred to fresh medium at 1–2 week intervals. The growth of cell suspensions during maintenance was evaluated with respect to inoculum density, medium sterilization and carbon source. Consistently healthy cultures were only obtained following weekly subculture in fresh medium. Suspension cultures could facilitate the *in vitro* manipulation of avocado by somatic hybridization since both *Persea* species are resistant to *Phytophthora* root-rot.

P-2056

Somatic Embryogenesis and Plant Regeneration from Cotton Anther Culture. B.H. ZHANG, R. Feng, F. Liu and X.L. Li. Cotton Research Institute, Chinese Academy of Agricultural Sciences, Anyang Henan 455112, China. E-mail: zbh68@hotmail.com

The embryogenesis and plant regeneration from cotton anther culture were described first in this paper.It was different among various species of Gossypium genus on the induction of anther callus, species were displayed from easy to difficult to induce callus: G. arboreum L. > G. hirsutum L. > G. barbadense L. > wild species, heterosis of callus induction was not showed in interspecific hybrid. The induction and growth of cotton anther callus were effected by hormones and sugar souces. Wild G. klotzschianum Anderss anthers could directly produced embryogenic callus on MS supplement with 0.1mg/L 2,4-D and 0.5mg/L KIN, but G. hirsutum L. could not,it could obtain embryogenic callus only through the selection with the method of starvation too.High frequency subculture was disadvantageous to the induction of embryogenic callus. Genotypes was one of the key factors on embryogenesis and plant regeneration from cotton anther callus. Embryos and regenerative plants were obtained only from Lumian No. 6, Coker 201 and Siokral 1–3 three varieties of Gossypium hirsutum L. and a wild species G. klotzschianum Anderss in 83 genotypes in our experiment. It was different among various sugar souces to induce embryogenesis of cotton anther callus was different, amylum was easiest,the follow was maltose and sucrose,glucose and lactose were vary difficult to induce embryogenesis. In suitable culture condition,microspores in anther culture could be divide into two cells,more cells or smell cell group, and then formed haploid callus.Both of haploid and hiploid cell existed in cotton anther culture, the ratio of haploid cell was 10.0% or so.Some of regenerative plants were haploid plants.

P-2058

Factors Affecting Induction of Somatic Embryogenesis in Velvetleaf (*Abutilon theophrasti*). Y.L. KLEIN and D.A. Steen. Biology Department, Andrews University, Berrien Springs, MI 49104. E-mail: yolanda@andrews.edu; steen@andrews.edu.

Seed pods of *Abutilon theophrasti* were collected from 10 different sites in Berrien County, MI over the course of one summer. Following disinfestation, immature zygotic embryos of various developmental stages were aseptically removed from the pods and placed in liquid or on gelrite-solidified MS induction media containing 4.5 uM 2,4-D and 20.0 uM silver thiosulfate. Embryos were either left on the induction media or transferred to hormone-free media after 28 days. Somatic embryos appeared most often on late heart or torpedo stage zygotic embryos with 21% and 38% respectively for induction media and 10% and 22% for embryos transferred to hormone-free media. Twinning of zygotic embryos was a common observation (60%). We found that the best indicator of zygotic embryo developmental stage within the seed pod was a combination of spine diameter, pod diameter and days postanthesis. No differences in somatic embryogenetic potential were found between explants from various collection sites or between different ages of donor plants.

P-2059

Somatic Embryogenesis and Plant Regeneration in Typha Aungustifolia (Narrow Leaf Cattail). S.D.ROGERS and K.S.Sarma. Bioscience Department, Salem-Teikyo University, Salem, WV 26426. E.mail: ROGERS@SALEM-TEIKYO.WVNET.EDU

Callus was induced from in vitro germinated seed- lings cultured on Murashige and Skoog(MS) medium supplemented with one of the following auxins, 2,4-D, Dicamba, or Picloram. Two months old callus produced somatic embryos. The somatic embryos were creamy white in colour and starch rich as observed by I_2-KI stain. Upon transfer to cyto- kinin supplemented medium somatic embryos were matured and could be germinated. The plants were successfully established in the greenhouse and subsequently in wetlands. All plants showed normal phenotype. The effect of auxins, and culture environment on somatic embryogenesis and plant acclimitization will be discussed.

P-2060

Analysis of Antibody-Binding Site of Rice Allergen RA17 with Human Monoclonal Antibodies. H. SHINMOTO, T. Kimura, K. Yamagishi and M. Suzuki. Tohoku National Agricultural Experiment Station, Arai, Fukushima 960-2156, Japan. Email: shinmoto@affrc.go.jp

To analyze the structure of food allergens, we planned to obtaine human monoclonal anbitodies to food allergens. Our method employed the immortalization of human B cells with Epstein-Barr virus (EBV) followed by cell fusion with mouse myeloma cells to establish stable human-mouse hybridomas. We obtained 2,202 multi-clone immortalized human B-cell library stocks from seven healthy donors by transforming peripheral blood B-cells with EBV. The library contained immortalized human B-cells secreting antibodies against rice, soybeans, peanuts, wheat, milk proteins and egg-white proteins. Five immortalized B-cell lines secreting anti-rice allergen antibodies were fused with mouse myeloma cells and human-mouse hybiridomas secreting human antibodies were established. Antibodies secreted from the hybridomas reacted rice major allergen molecules and analysis with allergen peptides synthesized on multi-pin apparatus revealed a binding sequence of the allergen protein RA17. The antigenic determinant was located in C-terminus region of the allergen protein. We concluded that our immortalized B-cell library developed here would be a powerful tool for analyzing allergens.

P-2061

In Vitro Propagation of Two Zizphus Species; Z. Spina christi and Z. Muratiana. S. AL-MAZROOEI and P. Ramos. Department of Biological Sciences, Faculty of Science, Kuwait University, B.O. Box 5969, Safat 13060, KUWAIT. E-mail: mazrooei@kuc01.kuniv.edu.kw

Zizphus species are heat and drought tolerant plants. They are different from each other by the size and shape of the fruits and leaves. Most parts of these plants are reported to be good sources of nutritional and medicinal compounds. Very few reports have been published on in vitro propagation of zizyphus species and to our knowledge this is the first one on Z. *spina christi*. Micropropagation of Z. *spina christi* and Z. *muritiana* was achieved through axillary bud culture from mature plants and from young seedlings. Explants were cultured on MS medium supplemented with a combination of different concentrations of BAP and IBA respectively, as follows: (1.0 mg l^{-1} + 0.05 mg l^{-1}), (0.2 mg l^{-1} + 0.01 mg l^{-1}), (0.6 mg l^{-1} + 0.005 mg l^{-1}), (0.2 mg l^{-1} + 0.005mg l^{-1}), (0.05 mg l^{-1} + 0.1 mg l^{-1}). Bud break and shoot elongation was successful at all concentrations tested with a maximum response with the third combination of growth regulators. For the two species incubation was at 30C° enhanced shoots. Elongation more rapidly than incubation at 25C°. Rooting of the shoots was achieved on MS medium supplemented with 1 mg l^{-1} IBA. In vitro plants were transferred to the green house successfully after

P-2062

Micropropagation of *Vaccinium cylindraceum* Smith (Ericaceae), an Azorean Endemic Species. M. J. PEREIRA. Department of Biology, University of Azores, Apartado 1422, 9501-801 Ponta Delgada Codex, Portugal. E-mail: pereira@alf.uac.pt

Micropropagation techniques were applied in the conservation of genetic variability within the endangered, natural populations of *Vaccinium cylindraceum* Smith, an ericad endemic to the Azores archipelago. Micropropagation of plant material with origin in three different islands was achievable in the Zimmerman and Broome-1980's medium using: a) nodal explants from wild-grown mature plants, b) nodal explants from new shoots developed *ex situ*, and c) seedling explants. The substitution of benomyl (12%, 30 minutes) by 'Plant Preservative Mixture' (0.4%, 30 minutes), in the disinfection procedure of field collected explants, resulted in a significant increase of visible contamination. When initiating cultures, the use of nodal explants from shoots of the year with origin in epicormics significantly increased the percentage of developed shoots with juvenile characteristics. In the initiation stage the use of a lower temperature (15°C instead of 22°C) and a lower saccharose concentration (2% instead of 3%) significantly improved the number of reactive buds. When initiating cultures with nodal explants, increasing concentrations of 2iP resulted in a heterogeneous response according the population of origin of the explants and the month of harvest. When initiating cultures with seedling explants, increasing concentrations of 2iP produced increasing numbers of shoots per explant and decreasing lengths for each shoot, while for nodal explants with origin in adult shrubs, this 2iP mode of action is only visible in the produced shoots that have attained some juvenile characteristics. In the initiation stage, the number of reactive buds from adult shrubs nodal explants, significantly increased in the media supplemented with: 73.8μM 2iP + 11.42μM IAA and 73.8μM + 4.92μM IBA, while zeatin and NAA were useless in the tested concentrations. 2iP suppression in the culture medium, enabled the shoot elongation and, for some of the produced shoots with seedling origin, *in vitro* rooting. *Ex-vitrum* rooting and acclimatization stage were successfully performed in Jiffy7(tr) pellets.

P-2063

Seed Germination and In vitro Propagation of Sucupira Branca [*Pterodon pubescens (Benth.) Benth.*], a Medicinal Plant. J.E.B.P.PINTO; M.C.F.Coelho; O.A.Lameira; E.J.A.Santiago and F.G.Silva. Laboratory Tissue Culture, UFLA Cx.P.37, Lavras, MG, 37200-000. email: jeduardo@ufla.br

Pterodon pubescens (Benth.) Benth. is a essence native to Brazilian cerrados, reaching up to 16 meters in height. The oil of fruit is very enjoyed in folk medicine in sore and rheumatic infections, it protects from cercaria infections. The wood posses high natural resistence to rotting, being regarded as one of the most resistent woods for railroad sleepers. The objectives of the present work were to identify the best in vitro and ex vitro germination conditions for the seed, embryo and embryonic axes of the seedlings development, in addition to determinating a methodology for in vitro multiplication. Experiments were conducted aiming at the achievement of in vitro germination by means of the use of modified MS basic medium, in liquid medium, different gelifyings, caps and light conditions the establishment of nodal segments were studied by employing the MS and WPM basic medium. In the multi-sprouting experiment, four different combined concentrations of ANA and BAP were tested. The best type of cap for embryo germination was the one of aluminum paper mould together with liquid medium the nodal segments were best established in WPM medium. Up to 7,5 shoots/segment were obtained, with the nodal segment, inoculated at the horizontal position in the MS culture medium with half the concentration of salts, supplemented with 0,5 mM BAP.

P-2064

Multiplication Strategies for *Hypericum foliosum* Aiton, an Endemic Azorean Species. Graciete Belo Maciel and MONICA MOURA. Departamento de Biologia, Universidade dos Açores. Apartado 1422, 9501-801 Ponta Delgada (Açores) Codex, Portugal. Email: maciel@notes.uac.pt, moura@notes.uac.pt

Hypericum foliosum Aiton, is a beautiful Azorean endemic species whose populations currently yield a low number of individuals in many of the archipelago islands. In order to find an effective *ex situ* multiplication strategy for this plant several studies were carried out. During a 3 years span the germination capacity of its seeds was tested. The essays took place under a continuous temperature of 15 °C and 10–20 °C alternate, for a period of 8 hours light and total darkness. In alternate temperature tests the light period was made to coincide with the highest temperature. The species showed a positive photosensitivity and germination percentage of 67% under a continuous 15 °C temperature. A short dormancy was detected in seeds with 3 months of conservation. At the end of 15 and 25 months of storage new essays were done and the results obtained showed a progressive reduction in the plant's germination capacity. The first micropropagation studies carried out revealed that the best medium to cultivate *Hypericum foliosum*'s single node cuttings was Côrte & Mendonça (1985), supplemented with 0.4 µM N6-benzyladenine (BA) and 2.6 µM a-naphthaleneacetic acid (NAA) + 4.4 µM BA, in the initiation stage and 0.4 µM BA, in the elongation stage. Regarding culture multiplication, 0.4 µM BA, in the initiation stage and 2.6 µM NAA + 4.4 µM BA, in the initiation and elongation stages, proved to be the most efficient concentrations. The acclimatization stage was also successfully performed in Jiffy 7(r) pellets. To fine tune the composition of the culture medium, several tests were then run using different quantities of sucrose. Namely, 5, 10, 20, 30 and 40 g/l. A higher differentiation and multiplication rate was achieved in a 20 g/l concentration, which also produced longer shoots and the highest percentage of rooting. The pH influence in *Hypericum* cultures' performance was also tested using 3 different values: 4.8, 5.8 and 6.8. Better differentiation and elongation values were achieved in pH=5.8, as also was the percentage of rooted explants. Regarding multiplication, 5.8 also produced the best results.

P-2065

Chemically Induced Resistance of *Carica Papaya* against *Phytophthora Palmivora*. Y. JUDY ZHU[1], Maureen Fitch[2], Stephen Ferreira[3] and Paul Moore[2]. [1]Hawaii Agriculture Research Center, Aiea, HI 98701, [2]USDA, ARS, Aiea, HI 96701, [3]University of Hawaii, Honolulu, HI 96822. E-mail: jzhu@harc-hspa.com

Acquired resistance is an inducible defense mechanism exhibited by many plants that provides protection against a broad range of pathogens. Systemic acquired resistance (SAR) has been induced by treatment with chemical substances such as salicylic acid (SA) and benzo(1,2,3)thiodizaole-7-carbothioic acid S-methyl ester (BTH) in both dicotyledenous and moncotyledonous plants. We are exploring the possibility of using BTH-induced SAR as an alternative approach for control of the root rot and fruit rot diseases caused by *Phytophthora palmivora*. Here we report that in tropical fruit papaya, *Carica papaya*, chemical treatments with SA and BTH can induce SAR against *Phytophthora palmivora*. Young papaya plants pretreated with 15 mM SA showed 35–50% fewer lesions and smaller infected areas two weeks after inoculation with *P. palmivora* than similar plants without SA treatment. BTH increased papaya plant resistance to *P. palmivora* at concentration as low as 1.0 mM. BTH at this concentration exhibited slight toxicity to the papaya seedlings but gave complete protection against *P. palmivora* whereas control plants treated with water showed over 70% mortality 5 days after inoculation. Studies on the effectiveness of a range of concentrations of BTH will be reported. Enzyme activities of the pathogenesis-related proteins chitinase and beta-1,3-glucanase increased more than six-fold following BTH treatment indicating that BTH is acting as a chemical inducer of SAR in papaya.

P-2066

One-step, *in vitro* Acclimatization of Carnation using a Mist Reactor. M.J. CORRELL and P.J. Weathers. Dept. of Biology/Biotechnology, Worcester Polytechnic Institute, Worcester, MA 01609. E-mail: mcorrell@wpi.edu

Mist reactors offer a variety of benefits when compared to conventional micropropagation techniques: the gas phase surrounding the plant tissue can be readily manipulated, large quantities of plants can be cultured in a single vessel, the liquid medium can be regulated throughout plant development, and they can be easily automated. An acoustic window mist reactor was used for *in vitro* culturing and subsequent acclimatization of *Dianthus caryophyllus* L. Plant nodes were cultured for five weeks in either the mist reactor or GA7 culture boxes (Magenta™), containing or lacking a 0.2 <FONT FACE=Symbol>m</FONT>m filter-vent in the lid. For plants grown in the reactor, the medium feed rate or misting cycle increased over the culturing period from 2 to 4 to 10 minutes of misting on per hour, for weeks 1, 2, and 3–5, respectively. In addition, the last week of *in vitro* culture within the reactor included a stepwise reduction in relative humidity from 99% to 70% Rh using dried, ambient air to flush the headspace surrounding plant tissues. Plants from both the reactor and corresponding GA7 boxes were transferred to the greenhouse for five weeks without additional acclimatization. Plant survival was highest for plants grown in the mist reactor (89% survival) compared to plants grown in either GA7 boxes (50% survival) or GA7 boxes with filter-vents (81% survival). Survival correlated with low levels of hyperhydration. These results show that by careful manipulation of the environment, one-step acclimatization can be achieved using a mist bioreactor.

P-2067

Development of Interspecific Hybrids in Oil Seed *Brassicas (Cruciferae)*. V. RAJA and I. Ahuja. Brassica Biotechnology Group, National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, Pusa, New Delhi, 110012 INDIA. E-mail: dnavarsha70@hotmail.com

Since brassicas is an important source of major oilseed crop, much emphasis has been given to widen its gene pool. As the existing genetic variability is limited, a wide hybridization program has been used extensively to create variability. One of the reliable means of pollination control for hybrid varieties is the use of cytoplasmic-genic male sterility systems (CMS). With the objective of transferring CMS from wild type genotypes to cultivated ones, crosses were made to obtain interspecific hybrids. Hybrids were obtained by excising ovaries in in-vivo, after 4–5 days of pollination and were inoculated on Murashige and Skoog's medium of Casein Hydrolyasate (500 ml^{-1}) and CMS lines were obtained. After backcrossing to cultivated types, these CMS lines can be further exploited for the production of commercial hybrid seed after obtaining suitable restorer and maintainer lines

P-2068

DETECTION AND ELIMINATION OF *VERTICILLIUM* INFECTIONS OF MINT. NAN WANG, Department of Horticulture, Oregon State University, Corvallis, OR 97331 and Barbara M. Reed, USDA-ARS National Clonal Germplasm Repository, Corvallis, OR 97333-2521 USA. Email: reedbm@bcc.orst.edu

Roots of mint greenhouse plants and shoot cultures were inoculated with *V. dahliae* conidial suspensions to study symptom development, detection, and elimination of the fungus. There were significant differences in the appearance of control and infected shoot cultures at all infection levels for the four cultivars tested. Disease symptom ratings were proportional to the *V. dahliae* inoculum density. Infected shoot cultures were stunted at and above 10^3 conidia/ml. 'Scotch Spearmint' stems became thicker and split at 10^3 to 10^5conidia/ml. *V. dahliae* was reisolated from infected shoot cultures at all levels of inoculum and no fungus could be isolated from any control cultures. *Verticillium* infections were easily detected by plating stems on potato dextrose agar. Meristems (0.5 to 15 mm) from infected *in vitro* and greenhouse plants were isolated and screened for fungus. Meristems of 3–5 mm length produced the most *Verticillium*-free cultures. The effectiveness of meristem tip culture also depended on the initial inoculum level and the cultivar. Meristems from *in vitro*-infected spearmint cultivars at 10^2 and 10^3 conidia/ml were 100% *Verticillium* free. However, only 42% were *Verticillium*-free for 'Black Mitcham' and 54% for 'Todd's Mitcham' peppermints. Infected greenhouse plants produced *Verticillium*-free cultures from 79% of 'Black Mitcham' and 90% of 'Todd's Mitcham' meristems. This study shows that *V. dahliae* can be easily detected both *in vitro* and *in vivo*. *Verticillium*-free plants can be produced from infected greenhouse or *in vitro* meristems 3–5 mm in size.

P-2069

Optimization of an in vitro bioassay for wheat diseases. M.M. Salgado, A. PELLEGRINESCHI, M. Mezzalama, S. McLean, and D. Hoisington. Applied Biotechnology Center, CIMMYT. Email A.Pellegrineschi@cgiar.org

Alternaria triticina, *Fusarium graminearum*, *Pyrenophora tritici-repentis* (*Helmintosporium*), *Pythium* sp., and *Rhizoctonia* were tested on the basis of being a representative group of important fungal pathogens in order to establish a reliable bio-assay system to test eventual fungal resistance in transgenic plants. The inoculum were prepared on V-8 agar medium (in petri dishes) for the case of *P. tritici-repentis* and *Pythium*) sp., on PDA (Potato Dextrose Agar) medium for the other fungi. The cultures were incubated at room temperature for 7–10 days in a culture chamber with a constant standard illumination. The suspensions of conidia or micelia were prepared on sterile distilled water with a few drops of Tween 20 and scrapings of the fungi culture. The inoculum was homogenized by vortexing the suspension for a few seconds. The concentration used for the conidia solution was adjusted to 10,000 conidia/ml. Fresh leaf samples, from adult plants (heading stage) were sterilized and then dipped in the inoculum suspension. The inoculated leaves were then transferred to water agar medium (1% agar), in 8-well rectangular multi-dishes, at room temperature. The level of resistance of the material to these pathogens was evaluated after 4–7 days after inoculation. We believe that the routine use of this protocol (fast, reliable and inexpensive) could allow the easy identification of resistant/tolerant plants to these diseases. In addition this test, due to its simplicity can be useful for early screening of large number of individuals, as required for transgenic plant screenings.

P-2070

Cryopreservation of Temperate and Tropical Crops by Encapsulation-Vitrification Protocol. D. HIRAI and A. Sakai [1]. Hokkaido Plant Genetic Resources Center, 363-2, Minami-takinokawa, Takikawa, Hokkaido, 073-0013, JAPAN. E-mail:hiraidai@agri.pref.hokkaido.jp. 1) 1-5-23, Asabu-cho, Kita-ku, Sapporo, Hokkaido, 001-0045, JAPAN. E-mail: asakai@mxu.meshnet.co.jp

Alginate-coated meristems from in vitro-grown axillary buds of potato (14 cvs.), mint (3 species), cassava (2 cvs.), in vitro-grown apical buds of Chinese yam (2 species), lily (14 cvs.) and meristematic clumps of strawberry (6 cvs.) were successfully cryopreserved by encapsulation-vitrification. In this method, encapsulated meristems following preconditioning were osmoprotected with a mixture of 2 M glycerol plus 0.4 or 0.8 M sucrose for 60 to 90 min. They were then sufficiently dehydrated with a highly concentrated vitrification solution (PVS2 solution) for 2 to 4 h at 0 °C prior to a plunge into liquid nitrogen. Successfully vitrified meristems of all tested crops developed shoots in 2 to 4 weeks after plating without intermediary callus formation. With some modifications of preconditioning specimens, such as cold-hardening, preculture with sucrose enriched medium, and the incubation procedure after cryopreservation, this protocol has been applied to a wide range of plants (46 species or cultivars) of both temperate and tropical origins. The average rate of recovery growth of all plants tested amounted to nearly 65%. It was also confirmed that no difference in PCR products was observed in RAPD analysis using 200 primers between cryopreserved and non-treated control plantlets of potato, lily, Chinese yam and cassava. Thus, this encapsulation-vitrification protocol appears promising for cryopreservation of both temperate and tropical germplasm.

P-2071

Cryopreservation of Somatic Embryo of Papaya (*Carica papaya* L.) by Vitrification. T.G.Lu[1] and H.Takagi[2].[1]Dept. of Plant Sciences, Univ. of Arizona, Tucson 85721, AZ, USA. [2] Japan International Research Center for Agricultural Sciences, 1-2 Ohwashi, Tsukuba, Ibaraki 305-8686, Japan. E-mail: takagiw@jircas.affrc.go.jp

Papaya (*Carica Papaya* L.) is one of the most widely grown fruit crops in the tropics and subtropics.Seeds are mainly used for multiplication but can be conserved only for about a year even under the favorable condition. Therefore, development of long-term conservation method for germplasms is necessary. Recent progress in biotechnology, i.e. genetic transformation, utilization of artificial seeds, also has increased the interests in cryopreservation to maintain regeneration capacity and genetic stability of selected clonal materials.Somatic embryo clumps of papaya (cv. Sunrise solo) were successfully cryopreserved by vitrification method. Somatic embryo clumps consisting of adventitious embryos, younger than torpedo shape, were the best candidate among embryos at different developmental stages tested. Small pieces of embryo clumps were treated with a mixture of 1.5M glycerol, 0.4M sucrose and 5% DMSO for 25min. at 25°C, and were then sufficiently dehydrated with a highly concentrated vitrification solution, PVS2, for 25min. at 25°C before being into LN_2. Vitrified and warmed embryo clumps remained normal yellowish color and resumed growing within one week. They developed into mature embryos without forming secondary embryos and calluses. More than 70% of these mature embryos germinated and developed plantlets. This cryopreservation protocol for somatic embryo of papaya resulted in high survival rate. The procedure was also simple and considered to be promising for long-term preservation of papaya germplasm.

P-2072

In Vitro Propagation of Edible Ginger, *Zingiber offincinalie* as Influenced by Bud Dormancy and Autotrophy. M.J. Tanabe and K. Okuhara. University of Hawaii at Hilo, College of Forestry and Natural Resource Management, 200 West Kawili St., Hilo, Hawaii, 96720-4091. Email: mtanabe@hawaii.edu

A bacterial disease caused by *Pseudomonas solanacearum* has caused severe crop losses in the State of Hawaii. This disease can be readily spread by rhizome pieces used for conventional propagation. Growers are addressing this problem by using in vitro cultured plants as clean starting material but laboratories have reported inconsistent in vitro growth responses. This study demonstrated that the dormant state of the rhizome buds contributed greatly to this condition. Fifty per cent of the buds obtained from rhizomes harvested during October produced shoots after 3 weeks. No shoots were produced from rhizomes harvested during December and January for the same observation period. There was also a relationship between bud ranking (locations of bud on rhizome) and dormancy. Proximal buds (nearest to an attached older rhizome) broke dormancy 5 weeks after initial explanting and distal buds (furthest from an attached older rhizome) did likewise 8–10 weeks after explanting. Transfer of dormant buds into liquid Murashige and Skoog (M&S) medium supplemented with 9(mu)M 6-Benzylaminopurine (BA) and 570 (mu)M Gibberellic Acid (GA) resulted in 80% of the buds breaking dormancy 3 weeks after treatment. Treatment with BA only resulted in 40% bud break after 10 weeks. In vitro plantlets were able to grow in liquid M&S supplemented with 9(mu)M BA and no sucrose. Cultures containing this medium showed a 100 ppm reduction in carbon dioxide level between 7: 00 am and 12:00 pm. Cultures with M&S plus 3% sucrose showed very little change in carbon dioxide level during this same observation period. It is probably that the plantlets grown in the medium without sucrose had achieved autotrophy. These plantlets also had little difficulty adapting to the ex vitro environment.

P-2073

Transgenic Kentucky Bluegrass (*Poa pratensis L.*) Plants Obtained by Microprojectile Bombardment of Embryogenic Callus. C. GAO and L. Hansen. DLF-Trifolium A/S, Research Divsion, Højerupvej 31, DK-4600, Store Heddinge, Denmark. E-mail: CXG@DLF.DK

Kentucky bluegrass (*Poa pratensis L.*) is an important turf and forage grass, but the apomictic mode of reproduction makes cultivar improvements by conventional breeding difficult. This is the first report on the regeneration of green plants from transformed embryogenic callus in this species. Resistance to bialaphos, a broad-spectrum herbicide, was introduced into *Poa pratensis cv.* Geronimo by microprojectile bombardment-mediated transformation. The plasmid DNAs containing both the bialaphos resistance (*bar*) gene driven by maize ubiquitin promoter and an intron region, and the beta-glucuronidase (*gus*) gene controlled by rice actin1 promoter and an intron, were transformed into embryogenic callus of Geronimo by microprojectile bombardment. Transformed embryogenic callus was grown on bialaphos-supplemented callus induction medium and subsequently shoot regeneration medium. Transient and stable GUS expression was observed on transformed callus. A total of 14 individual plants were regenerated. GUS and chlorophenol red (CR) assays were used to identify transformed plants. Integration of transgenes was confirmed by PCR and Southern hybridization. Herbicide (Basta) tolerance up to 5000 ppm of these plants was demonstrated in the greenhouse trials.

P-2074

CALLUS INDUCTION AND ORGANOGENESIS IN WHEAT HYBRIDS AND AMPHIPLOIDS. TYANKOVA N., Dryanova A., Zagorska N., Dimitrov D. Institute of Genetics, Bulgarian Academy of Sciences Sofia 1113, Bulgaria. Email: ddryan@argo.bas.bg

The behaviour of immature embryos of different wheat hybrids and amphiploids grown on callus inducing medium enriched with various PEG 6000 MW concentrations for drought selection was studied. The amphiploids Triticum aestivum/Agropyron elongatum (AABBDDEE), 2n =56, Triticum durum/ Agropyron elongatum (AABBEE), 2n =42 and T.durum/ Haynaldia villosa (AABBH^{v}H^{v}), 2n=42, as well as the hybrid T. aestivum/ Agropyron intermedium—Wheat-WheatGrass Hybrid 31 (WWGH 31), 2n =42 were used as initial material. Callus formation occurred in all genotypes. No significant variations among the genotypes were observed. Only the amphiploid T. durum/A. elongatum differed by the lower percentage of calli (55.5% compared to 71.9% and 69.3% for the other genotypes, respectively), but it was of intermediate position according to embryogenic capacity. Most intensive callus induction and embryogenesis was manifested by the amphiploid T. durum/ H. villosa. This amphiploid showed the best results in rooting in PEG absence (64.0%) as well as in addition of 5% and 10% PEG (34.7% and 38.6%, respectively). Òhe results in WWGH 31 were similar except the lower embryogenic callus rate compared to T. durum/ H. villosa (38.6% and 54.3%, respectively) and plant development on medium supplemented with 10% PEG which was 20.9% and 38.6%, respectively. The selected regenerants were planted in pots and grown up in green house conditions.

P-2075

Plant Regeneration Through Somatic Embryogenesis from Leaf Tissue of *Odina wodier* Roxb., a Tropical Tree. S. PANDEY and V.S. Jaiswal. Laboratory of Morphogenesis, CAS in Botany, Banaras Hindu University, Varanasi-221 005, INDIA

An efficient plant regeneration system through somatic embryogenesis from leaf tissue of an economically important adult tropical forest tree of *Odina wodier* Roxb. has been achieved. Leaf disc explants (0.8 cm in diameter) prepared from the fresh leaves collected in the month of April and May were inoculated on 0.8 (w/v) agar-gelled MS (Murashige and Skoog, 1962) medium containing 2,4-D (2,4-dichlorophenoxy acetic acid) (1.0–5.0 mg/l) in combination with KIN (Kinetin) (0.01–1.0 mg/l) with 3% (w/v) sucrose. After 2–3 weeks of inoculation the leaf discs (80–85%) showed callus initiation (light yellow or cream coloured) on medium containing 5.0 mg/l 2,4-D + 0.01 mg/l KIN. These calli stopped their proliferation after 3 weeks of initiation if retained or subcultured on the same medium and became compact green and/or brown. However, the calli subcultured on MS basal medium proliferated, retained their cream colour, became shiny and friable. From such friable calli, shiny globular structures were produced within 8–10 weeks on MS basal medium. Different stages of somatic embryos (globular-, heart-, and cotyledonary-stage) were differentiated from these globular structures on the MS basal medium. Approximately 50% of the cotyledonary stage somatic embryos (>5mm in length) successfully converted into plantlets after 2–4 weeks on MS basal medium with 3% (w/v) sucrose.

P-2076

In Vitro Assay of 2,4-D Resistance Transgenic Cotton. BAO HONG ZHANG, Hong-Mei Wang, Fang Liu, Yun-Hai Li & Zheng-De Liu Cotton Research Institute, Chinese Academy of Agricultural Sciences, Anyang Henan 455112, P. R. China E-mail: zbh68@hotmail.com

2,4-D resistance plants of transgenic cotton (Gossypium hirsutum L.) were produced by using Agrobacterium tumefaciens containing a plasmid carrying the npt II and tfd A genes. An in vitro assay was performed to determine the sensitivity of seed germination and the growth of seedlings of transgenic and non-transgenic cotton to various concentrations of kanamycin and 2,4-D. The results indicated the kanamycin caused the cotyledons of non-transgenic plants turning ablio, but transgenic plants grew normally. Seed germination and seedling growth of non-transgenic plants were restrained remarkably by 2,4-D, but for transgenic plants lightly. Transgenic plants and non-transgenic plants can be distinguished remarkably by the use of 2mg/L 2,4-D. There was a high correlation between the response of seed germination and the growth of seedlings to kanamycin or 2,4-D, based on the germination ratio, ablio ratio, dry weight or fresh weight. On this basis, we developed a rapid method for identifying transgenic plants, which has been proved by field method. These findings will allow identification of cotton transformants at an early stage of plant development, saving time and improved cultivars with the resistance trait.

P-2077

Role of Suspension Culture in Scale-up of Somatic Embryogenesis in mango (*Mangifera indica* L.) cv. Amrapali. H. ARA, U. Jaiswal, and V.S. Jaiswal. Laboratory of Morphogenesis, CAS in Botany, Banaras Hindu University, Varanasi-221 005, INDIA.

Plant propagation through conventional methods in mango is very difficult. Rapid and large-scale multiplication of mango plant through tissue culture is one of the current programmes of plant biotechnology. The present paper describes an improved method of plant regeneration in one of the highly prized Indian mango cultivar through somatic embryogenesis by using cell suspension culture. The suspension culture was established with the embryogenic calli derived from nucellar tissue of monoembryonic hybrid mango cultivar 'Amrapali' in liquid medium containing B5 (Gamborg, et al, 1968) macronutrients, MS (Murashige and Skoog, 1962) micronutrients and organics, 400 mg/l L-glutamine, 6% (w/v) sucrose with different combinations of 2,4-D (2,4-dichlorophenoxy acetic acid) and KIN (kinetin). After 3 weeks of starting the suspension culture from 10 mg embryogenic calli approximately 850 mg to 1,650 mg calli were produced per culture, that was 3–5 times more in comparison to those obtained on semi-solid medium. Morphotypes of the embryogenic calli varied with growth regulator(s) used in the medium. The embryogenic calli suspended in the liquid medium containing 2,4-D (1.0 mg/l) in combination with KIN (1.0 mg/l) proliferated in the form of fine suspension of single cell to small aggregates of cells, whereas those suspended in liquid medium containing either 2,4-D or KIN proliferated in the form of compact irregular masses (0.5–2.0 mm in size). On plating, the fine suspension of single cell and cell aggregates were found more efficient for regeneration of somatic embryos in comparison to the compact masses as 205.46 $\pm$ 7.69 and 341.67 $\pm$12.56 somatic embryos/10 mg calli could produce from the fine suspension of cells and compact masses respectively. The somatic embryos (60–70% of total somatic embryos produced from these calli) matured and converted (approximately 50% of the total somatic embryos matured) into plantlets successfully. The *in vitro* raised plantlets were acclimatized and transplanted in soil.

P-2078

Plant regeneration through somatic embryogenesis in root derieved callus of elephant tusk cactus (Coryphantha elephantidens (Lem.) Lem.). BRIJMOHAN S. BHAU* and A. K. Wakhlu#, *Laboratory of Cellular & Molecular Cytogenetics, Department of Botany, University of Delhi, India 110 007. #Plant tissue Culture Laboratory, Department of Botany, University of Jammu, Jammu-180 006 J&K, INDIA

Rare and endangered plants such as cacti, usually present limited reproductive capacities and very slow growth rates. They also comprised of very important ornamental plants, which are not easily available due to slow rate of propagation by conventional methods. The use of in vitro culture methods can over come these difficulties and provide large number of plants in a shorter time. A protocol was developed from root explants of in vitro raised plantlets as explants, and Murashige and Skoog media with the synthetic growth regulators 2,4-dichloroacetic acid, 6-benzylaminopurine and naphthleneacetic acid. Plant development via somatic embryogenesis from callus culture involved multiple stages; these include the induction of embryogenic callus, development of embryos, conversion of embryos into plantlets. Growth regulator concentration and type varied with each step. Effect of ABA, gibberlic acid and carbon source was found to be significant for embryo maturation. The somatic embryos were capable of germination and successful transfer to the field.

VT-2000

Assay Validation for Mycoplasma Screening Using Gen-Probe MTC-NI Detection System. S. NADOLSKI, J. Demers, M. Griffin, and P. Ozker. Quality Control Microbiology, Genetics Institute, Andover, MA 01810. Email: snadolski@genetics.com

Mycoplasma contamination is a serious event in cell culture lines, potentially causing adverse effects on cellular metabolism. Mycoplasma lack cell walls, and do not Gram stain. They are not detected visually since mycoplasma do not produce turbidity. These factors make it difficult to detect mycoplasma with traditional methods. Regulatory agencies require that all production cultures are free of adventitious agents making it critical to screen for mycoplasma in the biopharmaceutical industry. The Gen-Probe Mycoplasma Tissue Culture NI (MTC-NI) Rapid Detection System is used for the detection of mycoplasma contamination in cell culture samples. The MTC-NI system employs nucleic acid hybridization, using a single-stranded, chemiluminescent labeled, DNA probe which is complementary to the rRNA of the mycoplasma. The target organism's rRNA combines with the labelled DNA probe to form a stable DNA:RNA hybrid, which can be measured with a Leader 50 luminometer within two hours.The MTC-NI System has been evaluated for the screening of mycoplasma in the GMP Chinese Hamster Ovary (CHO) cell process at Genetics Institute. Protocols were conducted to validate the system and determine its comparability to the current assay using the Gen-Probe TC isotopic system. The specificity, limit of detection and robustness of the assay were addressed. The time, temperature and vortexing were varied to determine the robustness of the system. The Gen-Probe MTC-NI and the Gen-Probe TC assays exhibited matching results per protocol guidelines, which indicated >95% confidence level of agreement between the two assays. The assay was unaffected by varying the factors in the robustness test. Gen-Probe and CORE Cell Culture Facility, University of California at San Diego provided supporting data. The Gen-Probe MTC-NI Mycoplasma Detection System was validated and determined to be suitable for its intended use for screening for mycoplasma from GMP production cell cultures.

VT-2001

Hydrostatic Pressure Induced Interleukins and Cytokines Production by Normal Human Dermal Fibroblasts. S. KOYAMA, S. Fujii and M. Aizawa. Japan Marine Science and Technology Center, 2-15 Natsushimacho, Yokosuka 237-0061, Japan. Email: skoyama@jamstec.go.jp

The cellular responses have been recognized as the protective action of cells to adapt a change in environmental conditions. Here, we reported that cytokine production has been induced by extremely high hydrostatic pressure in normal human dermal fibroblasts. Normal human dermal fibroblasts were found to survive and were active in producing interleukin-6 (IL-6),−8, and monocyte chemoattractant protein-1 (MCP-1) under extremely high hydrostatic pressure, up to 70 MPa (0.1MPa = 1 atm), for 1 hr. The application of hydrostatic pressure triggered the expression and secretion of IL-6 and IL-8. Over 60MPa hydrostatic pressure application extremely enhanced IL-6 and IL-8 secretions (about 130 folds) without transcriptional enhancement. Induction of IL-1alpha, IL-1beta, and IL-12 mRNAs were appeared under high hydrostatic pressure condition. However, the fibroblasts did not produce IL-1alpha, IL-1beta, and IL-12 proteins. Extracellular accumulation of constitutively produced MCP-1 was down regulated in the pressure-applied fibroblasts in the absence of transcriptional repression. These results indicate that hydrostatic pressure induced interleukins and cytokines productions are also regulated at the post-transcriptional level.

VT-2002

Selective Induction of G-CSF in Macrophages by a Stimulatory Monoclonal Antibody: A New Signal Gateway Potentially Useful in Cytokine Therapy. Y. AOKI, S. Sha, H. Mukai and Y. Nishi. Laboratory of Life Science & Biomolecular Engineering, Japan Tobacco Inc., Aoba-ku, Yokohama, Kanagawa 227-8512, Japan. Email: yoshiko.aoki@ims.jti.co.jp

Granulocyte colony-stimulating factor (G-CSF) is one of the important cytokines that stimulates and activates segmented neutrophils in the bone marrow and in the periphery. G-CSF has a great advantage over the cytokine therapy for therapeutic use, since it has a selective activity to proliferate only neutrophils. Here, we demonstrate the identification of a stimulatory monoclonal antibody (mAb) that induces G-CSF in a mouse macrophage cell line. For this purpose, we used a sensitive assay system using luciferase as a reporter for G-CSF and an autoimmune mouse as the source of mAbs. One of the striking features of this mAb is its selective stimulatory effect toward the cells, whereas other stimulatory factors, such as lipopolysaccharide, fibronectin and vitronectin, showed pleiotropic induction. The induction was observed not only in the cell line, but also in normal peritoneal macrophage cell populations as measured by G-CSF mRNA. The antigen(s) were found to be distributed in normal peritoneal macrophages and in some established cell lines, such as monocytic/macrophage cell lines and pre-B leukemia cell lines. The mAb even recognized antigen(s) in a human promyelocytic leukemia cell line, HL-60 cells, when they were differentiated into monocytic cells. These results suggest that there could be a specific gateway molecule for signal transduction to induce G-CSF in macrophages.

VT-2003

Caco-2 High Throughput Screening Technologies: Pre-Grown Caco-2 24 Well Plate Product and the Use of a HTS Assay as a Rapid *In Vitro* Screen for Predicting Intestinal Drug Absorption. BRENDA F. KAHL, KAREN CLICK, Yong Hee Lee and Sandra R. Slivka. Cell and Molecular Biology, Trega Biosciences / Navicyte Inc., 9880 Campus Point Dr., San Diego, CA 92121. Email: bkahl@trega.com, kclick@trega.com

Purpose: Develop a high throughput screening assay for *in vitro* intestinal drug absorption using pre-grown Caco-2 cell monolayers. **Methods:** Pre-grown Caco-2 cell monolayers are shipped in BD/Falcon 24 well HTS insert plates and retain their functionality with respect to permeability of marker compounds. The monolayers are characterized by measuring transepithelial electrical resistance (TEER) as well as the permeability of mannitol, etoposide, glycylsarcosine (GlySar), and hydrocortisone under various shipping conditions. A high throughput screening (HTS) assay consists of removing the growth medium, the addition of a 50 uM test compound to the basolateral cell surface and a 2.5 hour incubation period. Caco-2 monolayer integrity is verified by TEER measurements pre- and post- dosing. Samples of the original dosing solution, donor (basolateral), and receiver (apical) side are analyzed by a high throughput radio-ligand binding assay. This assay was initially used to identify the compounds of interest for testing. Permeability values obtained from the bioassay were validated by LC-MS. This format allows maximum sensitivity with minimal compromise of monolayer integrity. Permeability data obtained from this assay may allow predictions of animal bioavailability. **Conclusion:** The cell monolayers survive both actual and simulated shipping conditions and can be used in a high throughput screening assay for predicting intestinal drug absorption to speed the process of drug discovery.

VT-2004

Selective expansion and non-radioisotopic assay of human natural killer cells. S. Watanabe, H. Harada, K. Saijo, T. Ohno. RIKEN (Inst. Physical. & Chem. Res.) Cell Bank, E-mail: satoru@rtc.riken.go.jp

We developed a simple method for human natural killer (NK) cell expansion with an adhesive target cell line HFWT that was derived from human Wilm's tumor. HFWT cells are scarcely expressing MHC-class I and class II molecules and therefore highly sensitive to human NK cells. Culture of peripheral blood mononuclear cells (PBMC) of healthy volunteers on HFWT cells for 10–16 days resulted in selective expansion of NK (CD3-CD56+CD16+) cells. PBMC, initially contained less than 15% of NK cells, were shared more than 50% of the population by NK cells after the culture. Cr-51 release assay has long been used as the standard method to quantify activity of NK cells. To avoid use of the radioactive substance in the assay, the adhesive HFWT cells were stained with crystal violet (CV) before and after the 4-hr incubation with NK population in which CD3-CD56+CD16+ cells shared 57%. NK cells were washed off after the incubation. The dye were quantified by the absorption at 570nm. At the effector/target ratio of 2 and 8, surviving target HFWT cells were 67% and 9%, respectively. More precise dose response curve corresponded reversively to that obtained by the standard Cr-51 release assay. After the 24-hr incubation, higher sensitivity of the killing was detected in the CV assay. These results suggest that human NK cells and non-radioisotopic CV assay are useful for further application in tumor therapy and/or health monitoring.

VT-2006

Two-dimensional Cell Blot Method. T. TERASAKI, Z. Yamaizumi and K. Tanaka. Pathol., Biol. and Radiobiol. Div., National Cancer Center Research Institute, Tsukiji 5-1, Chuo-ku, Tokyo 104-0045, Japan Email: tterasak@ncc.go.jp

In order to characterize functional proteins that induce changes in cells, a new method (Two-dimensional Cell Blot Method) was established. Fibronectin was partially digested with trypsin, two-dimensionally electrophoresed, transferred to a membrane and stained. After photocopied, the membrane was de-stained, blocked and baby hamster kidney cells (BHK-21) were cultured on the membrane over night. Cells on the membrane were fixed, stained and observed macroscopically or microscopically. Many protein spots to where BHK-21 cells attached and many protein spots to where those cells did not attach were observed. Immunological studies using antibody that had the activity to react with fibronectin cell binding domain showed that all the spot to where BHK-21 cells attached reacted with this antibody and all the spot to where those cells did not attach did not react with this antibody. Amino acid sequences of the proteins that are separated by two-dimensional polyacrylamide gel electrophresis and transferred to the membrane can be determined using amino acid sequencer. These showed that two-dimensional cell blot method is useful to characterize functional proteins that have activities to bind cells. This method must be also useful to characterize functional ptroteins that have activities such as to induce cell morphological changes, cell changes that can be detected by specific antibodies or cell death.

VT-2007

Role Of Matrix Metalloproteinases In Alpha-1-Proteinase Inhibitor Degradation By Neutrophil-Derived Proteases. W.J. BELLUCCI, E.J. Roemer, C.L. Ren and S.R. Simon, Dept. of Pathology, SUNY Stony Brook, Stony Brook, NY, 11794-8691. E-Mail: wbellucc@yahoo.com

Alpha-1 Proteinase Inhibitor (a1PI), the endogenous inhibitor of serine proteases is found proteolytically inactivated in diseases resulting from a recurrent inflammatory response such as Emphysema and Cystic Fibrosis. The goal of this study was to investigate the potential role matrix metalloproteinases (MMPs) might play in the degradation of a1PI. We lysed freshly isolated polymorphonuclear neutrophils (PMNs) in the presence or absence of 2.5mM Phenylmethylsulfonyl Fluoride (PMSF), a serine protease inhibitor. a1PI was incubated for 24 hours with normal or PMSF treated lysate with or without MMP inhibitors such as EDTA and 1,10 phenanthroline. SDS-PAGE showed degradation of a1PI by the normal lysate, which could be inhibited by MMP inhibitors. Degradation was also inhibited by incubation with PMSF or pre-treatment of PMN lysates with PMSF. Gelatin zymography showed active MMP 2 and 9 in the normal lysate whereas the inactive proforms were observed in PMSF pretreated lysates. Inhibition of in vitro extracellular matrix degradation by a1PI was enhanced by PMSF, EDTA and 1,10 phenanthroline. We conclude that MMP activity is involved in the degradation of a1PI and hypothesize that a serine protease activity may be required for this MMP activity. This study was supported by NIH (NIDER) DE-10985 (431-0264A)%llaGenex Pharmaceuticals, Inc., (431-6087A); USAMRMCDA-MD-1798-18560 (431-1241A); SUSB Center for Biotechnology (NYS)Science & Technology Foundation) (431-X324Q)

VT-2008

Evaluation of the Composition of the Extracellular Matrix Synthesized by Human Prostate Stromal Cells in Culture. E. SCOTTO-LAVINO, H.A. Sawka, S.R. Simon and E.J. Roemer, Department of Pathology, State University of New York at Stony Brook, Stony Brook, NY 11794-8691 E-mail: escottol@ic.sunysb.edu

Extracellular matrix (ECM) synthesized by R22 rat heart smooth muscle cells *in vitro* has been used in our laboratory to study the behavior of certain cell lines. We have adapted the culture protocols currently used for the rat cell system to establish the appropriate parameters for a human model based on commercially available normal human prostate stromal cells. These prostate cells grow very well in both a proprietary serum-free culture medium and in medium containing 5% FBS. With the addition of ascorbic acid, they will synthesize a stable, fibrous ECM. Culture medium containing either [^{3}H] proline and [^{35}S] sulfate or [^{3}H] fucose and [^{35}S]-cysteine/methionine is used to selectively radiolabel ECM components. Once the ECM has grown, the cells are removed and the matrix composition is analyzed by sequential enzymatic degradation. Individual enzymes: heparinase I & III, trypsin, chondroitinase, collagenase and human leukocyte elastase; are each incubated with the ECM for twenty-four hours, to extract individual matrix components. Supernatants from each step of the sequence are read by scintillation counting and analyzed to determine the relative proportions of components in the original, intact ECM. Enzyme-free control wells for each sample are incubated with the appropriate buffers and analyzed for spontaneous degradation of matrix, providing a relative measure of the ECM's physical stability and strength. By comparing matrix synthesized by cells from several different donors, we hope develop an *in vitro* human matrix system to use as a substrate for study of the degradative behavior of human prostate tumor cells. This study was supported by NIH(NIDER) DE-10985 (431-0264A); CollaGenex Pharmaceuticals, Inc., (431-6087A); USAMRMC DA-MD-1798-18560 (431-1241A); SUSB Center for Biotechnology (NYS Science & Technology Foundation) (431-X324Q) and URECA Grant #264900.

VT-2009

TYPE I COLLAGEN GENE EXPRESSION IN HUMAN UTERINE LEIOMYOMA CELL LINES, TAMMY WALLACE, Shelia McClure, Eniki Mack, Jameta Barlow, and Johnafel Crowe, Spelman College, Atlanta, GA 30314. E-mail: smcclure@spelman.edu

Fibroid tumors are benign tumors that originate from the myometrium layer of the uterus. They typically affect women during their reproductive years and more prevalent African-American women than any other ethnic group. Previous work in our lab has suggested that the level of type I collagen in the extracellular matrix is correlated with the growth potential of leiomyoma cell lines. The purpose of this work was to determine if the differences in type I collagen observed in the extracellular matrix are related to a change in type I collagen gene expression. The slow-growing HT1 cell line and the rapidly growing ST1 cell line were monitored for type I collagen expression utilizing Western blots. There were three distinct bands observed in the two tumors present at molecular weights of 295,000, 135,000, and 120,000. Expression of type I collagen is upregulated in the slower growing cell line HT1, which supports the hypothesis that increase in type I collagen in the matrix cell line HT1 occur as a result of a change in gene expression.

VT-2010

Enhanced Matrix Metalloproteinase (MMP)−2 Activity in Gingival Fibroblasts from Down Syndrome Patients. [1]T. KOMATSU, [2]M. Furue, [3]T. Kubota, [1]A. Miyagi, [4]E. Kubota. Department of [1] Dentistry for Special Patients, [2] Biochemistry, [3] Orthodontics, and [4] Oral & Maxillofacial Surgery, Kanagawa Dental College, Yokosuka Kanagawa 238-8580. Japan. E-mail: tomoko13@kdcnet.ac.jp

Objectives: To elucidate factors liable to periodontal disease in Down syndrome (trisomy 21), we studied mRNA expression and enzyme activity of matrix metalloproteinases (MMPs) in cultured gingival fibroblasts (GF) from Down syndrome patients and controls. Materials and Methods: Gingival tissue were biopsied from 9 patients with Down syndrome and 9 control patients at the time of tooth extraction. Specimens were primarily cultured in DM/F12 Medium supplemented with 10% FBS, and GF were subcultured in serum-free medium for assays. MMP mRNA expressions in the GF were determined using the semiquantitative reverse transcription polymerase chain reaction (RT-PCR). MMP activities in the conditioned medium were also measured by gelatin impregnated zymography. Result and Conclusions: The relative mRNA expressions of membrane-type1 MMP (MT1-MMP) and MMP-2 in GF from Down syndrome patients (D-GF) were constitutively augmented when compared with those of control gingival fibroblasts (C-GF). Production of the active-MMP-2 in D-GF was found to be significantly higher ($p < 0.05$) than that of C-GF at the protein level. These findings suggest that the high activity of MMP-2 in gingival fibroblasts could possibly play a role in gingivitis/periodontitis of Down syndrome patients.

VT-2011

Protein Kinase C Functions in TGF-beta-Induced Cellular Senescence Programs. Y. KATAKURA, Y. Tabira, T. Miura, N. Uehara and S. Shirahata. Graduate School of Genetic Resources Technology, Kyushu University, Fukuoka 812-8581, Japan. Email: katakura@grt.kyushu-u.ac.jp

We previously reported that TGF-beta triggers two independent senescence programs, replicative and premature senescence programs, in human lung adenocarcinoma cell line, A549 cells. A549 cells represses telomerase reverse transcriptase (hTERT) mRNA expression by the treatment of TGF-beta for 2 days and expresses senescence-marker of beta-galactosidase at pH 6.0 after the 7 days' treatment. The former indicates the induction of replicative senescence and the latter demonstrates that of premature senescence. Here we tried to identify the signaling cascade leading to induce cellular senescence by TGF-beta treatment. Among several inhibitors tested, D609, which is specific for phosphatidyl choline specific phospholipase C, completely blocked the TGF-beta-induced cellular senescence. Next we tested for the involvement of PKC in the TGF-beta induced cellular sencescence. Among the PKC inhibitors tested, calphostin C (specific for cPKC and nPKC) and Ro-31-8220 (specific for all types of PKC), but not Go6976 (specific for cPKC) specifically inhibit the TGF-beta induced cellular senescence. These results demonstrate that nPKC and/or aPKC functions in the TGF-beta induced cellular senescence programs. Now we have an attempt to identify the PKC isozyme functioning in the TGF-beta induced cellular senescence programs.

VT-2012

Hyperoxia Induces the Neuronal Differentiated Phenotype of PC12 Cells via a Sustained Activity of Mitogen-Activated Protein Kinase Induced by Bcl-2. S. KATOH, Y. Mitsui, K. Kitani and T. Suzuki. Radiation Safety Office, University of Tokyo Hospital, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8655, Japan, E-mail: skatohtky@umin.ac.jp

Reactive oxygen species (ROS) have been suggested as an induction signal for neuronal differentiation. This suggests that neuronal differentiation is induced by hyperoxia which is known to produce of ROS. Indeed, the incubation of PC12 cells under hyperoxia caused neurite extension. In these cells, amounts of differentiation-marker proteins increased. The effects of hyperoxia were inhibited by anti-oxidant reagents, suggesting the involvement of ROS. Further, artificial generation of ROS induced the same effects as hyperoxia. These suggest that ROS affects mitogen activated protein (MAP) kinase activity, which is known as a key enzyme of the signal transduction cascade for differentiation. A rapid increase in MAP kinase activity was observed under hyperoxia. Although the activity level then quickly decreased, activity higher than the control level was observed for 48 hours. PD98059, an inhibitor of MAP kinase, suppressed the hyperoxia-induced neurite extensions, suggesting the involvement of MAP kinase activity in the ROS-induced differentiation. While, ROS are harmful to cells and are known to induce apoptosis in neuronal cells. This suggests that the ROS-related mechanism of inducing differentiation is activated simultaneously with some type of apoptosis-inhibiting mechanism. Indeed, an expression of bcl-2, an apoptosis inhibitor, was elevated after culturing PC12 cells for 24 hours under hyperoxia. This bcl-2-elevation was not affected by treatment with PD98059, suggesting that this elevation did not directly induce neurite extensions under hyperoxia. However, the blockade of the bcl-2-elevation by an antisense oligonucleotide inhibited the sustained MAP kinase activity and neurite extensions under hyperoxia. Further, in PC12 cells highly expressing bcl 2, the sustained MAP kinase activity and neurite extensions under hyperoxia were enhanced. These results suggested that MAP kinase is activated via the production of ROS, and the subsequent elevation of bcl-2 expression sustains the MAP kinase activity, resulting in the induction of the neuronal differentiation phenotype of PC12 cells under hyperoxia.

VT-2013

Participation of Bcl-2 in Cellular Life Span. M. SASAKI, T. Kumazaki, M. Nishiyama, Takano, H. and Y. Mitsui*. Dept. Biochem. Biophys., Res. Inst. Rad. Biol. Med., Hiroshima Univ., 734-8553, and *Natl. Inst. Biosci. Hum. Tech., AIST, 305-8566, Japan. E-mail:kumazaki@ipc.hiroshima-u.ac.jp

Normal diploid cells have a limited life span in culture, which is measured by the number of population doublings (PD). Cellular lifespan may be, in part, determined by the rate of apoptosis. Bcl-2 is a well-known anti-apoptotic protein. Thus, Bcl-2 may take part in life span of a culture by preventing cell death. To examine this possibility, the level of Bcl-2 was reduced by adding anti-sense oligo-nucleotide against the bcl-2 gene to the culture medium. Under this culture condition, the fibroblast strain, MJ90, showed a shorter life span of about 12 PD from untreated control culture. To further confirm this result, we then introduced a plasmid, which highly expresses anti-sense bcl-2 RNA, into the MJ90 fibroblast and obtained 10 stable clones. These clones showed a decreased level of Bcl-2 and shorter life span of about 10 PD compared with that of vector alone controls. Thus, Bcl-2 participates in the length of life span, probably by preventing cell death triggered by oxygen radicals and/or other causes. Under conditions of depressed Bcl-2, cell death may be induced more easily, which causes the culture accumulate fewer population doublings before it reaches senescence. On the other hand, our study showed that the Bcl-2 level decreased with cellular aging of parental MJ90. However, senescent cells were more resistant to cell death induced by hydrogen peroxide. Thus, our data show that resistance of senescent cells to death is not caused by overexpression of Bcl-2, although this has been shown by others. In summary, Bcl-2 takes part in cellular life span, but not in resistance to death in senescent cells.

VT-2014

Subtractive Screening of Genes Involved in Cellular Senescence NORIHISA UEHARA, Yoshinori Katakura, Takumi Miura, and Sanetaka Shirahata. Graduate School of Genetic Resources Technology, Kyushu University, Fukuoka 812-8581, Japan. Email: nuno@grt.kyushu-u.ac.jp

The replicative life-span of normal somatic cells is strictly determined by a senescence program in which many gene products are thought to participate. Although several genes involved in telomerase regulation or cellular senescence have been identified, the whole picture of the senescence program is not fully understood. Thus, we attempted to identify the genes involved in cellular senescence, telomere maintenance and telomerase regulation through subtractive screening of cDNA libraries prepared from a human lung adenocarcinoma cell line A549 and its sublines named A5DC7, CK and AST-9. These cell lines have different phenotypes in terms of telomerase activity and telomere maintenance, and thus are thought to be useful for identifying the genes involved in cellular senescence and telomerase regulation. As a result of the subtractive screening, we identified 88 genes specifically expressed in either A549, A5DC7, CK or AST-9 cells. Sequences with high homology to several of our clones were found in the Genbank sequence database. Fifteen clones were demonstrated to be the cancer-related genes, and 7 clones were found to be the senescence-associated genes. However, the partial sequences of 28 of our clones do not have any significant homology to the sequences deposited in the Genbank database including the EST database, suggesting that these clones code for novel genes involved in telomerase regulation and cellular senescence.

VT-2015

Characterization of The 5'-Flanking Region of Human Telomerase Reverse Transcriptase Gene. T. MIURA, Y. Katakura, S. Nishimura, Y. Tabira, Y. Kotake, N. Uehara, K. Tashiro and S. Shirahata. Graduate School of Genetic Resources Technology, Kyushu University, Fukuoka 812-8581, Japan. Email: takumi@kyushu-u.ac.jp

Telomerase is a ribonucleoprotein enzyme composed of a template RNA and several proteins. Expression of the gene encoding the catalytic subunit of human telomerase (hTERT) is indispensable for the activation of telomerase in cancer cells. However, transcriptional regulation mechanism of hTERT gene has not yet been clarified. Here, we analyzed the structure and function of the 5'-flanking region of the hTERT gene. To obtain minimal promoter region of hTERT gene, various deletion fragments encompassing the hTERT gene promoter was inserted upstream of the luciferase reporter gene and transiently transfected into human cell lines of cancer cell lines and normal fibroblast. In endogenous hTERT-expressing cells such as cancer cells, the isolated hTERT promoter showed high levels of luciferase activity. Especially, a−286bp region (-286 to−44) had the most highly promoter activity. In comparison, the same hTERT promoter failed to work in normal cells. Thus, this region might contain essential elements for hTERT gene expression in cancer cells. Moreover, analysis of randomly prepared fragments revealed that enhancer elements were located at the region in 3500-1721 base pairs upstream from the translation start site. The region contains potential binding sites of various transcription factors such as c-Myc, AP-1, MZF-1, NF-1, estrogen receptor and GATA-1. We speculate that these transcriptional factors might regulate the promoter activity in hTERT gene.

VT-2016

Expression of Urokinase-type Plasminogen Activator mRNA by IL-1beta and TNF-alpha in Human Lung Microvascular Endothelial Cells. [1]K. KOBAYASHI, [1]K. Takahashi, [1]H. Yagyu, [1]T. Morisako, [1]K. Kishi, [1]R. Mikami, [1]O. Watanabe, [1]S. Oh-ishi, [1]H. Nakamura, [2]T. Hashimoto, [1]T. Matsuoka [1]Fifth Department of Internal Medicine, Tokyo Medical University, 3-20-1 Chuo, Ami, Inashiki, Ibaraki, 300-0395, Japan; and [2]Mito Saiseikai General Hospital, Ibaraki, Japan. E-mail: kimikot@tokyo-med.ac.jp

Urokinase-type plasminogen activator (u-PA) plays an important role in the process of tissue repair at inflammatory sites such as acute lung injury. When human lung microvascular endothelial cells (HLMECs) were stimulated with IL-1beta or TNF-alpha, the cells secreted the large amount of u-PA than that from unstimulated cells. In order to clarify the role of HLMECs in acute lung injury, the effects of IL-1beta and TNF-alpha on the expression of u-PA mRNA in HLMECs were examined using a competitive RT-PCR method. HLMECs were purely isolated from normal regions of lungs of patients undergoing resection for solitary lung tumors and cultured with medium 199 cotaining 20 % newborn calf serum, 10 ng/ml bFGF, 100 IU/ml penicillin, and 0.1 mg/ml streptomycin. IL-1beta or TNF-alpha (0.1, 1.0 and 10 ng/ml) was added to HLMECs and then the cells were incubated for 12 hours. Both IL-1beta and TNF-alpha significantly increased the expression of u-PA mRNA in HLMECs. HLMECs were also incubated for 4, 8 and 12 hours with 1.0 ng/ml of IL-1beta or TNF-alpha. The expression of u-PA mRNA increased significantly at 4, 8, 12 hours. These results suggest that at inflammatory sites HLMECs start to induce u-PA mRNA in an early stage in preparation for neovascularization and tissue remodeling.

VT-2017

Expression of MHC Antigens and Adhesion/Costimulation Molecules of Dendritic Cells from Human Blood During their Differentiation *In Vitro*. M. CHIRIVA-INTERNATI[1], F. Grizzi[2], G. Ceva-Grimaldi[3] and N.Dioguardi[2]. [1] Myeloma and Transplantation Research Center, Arkansas Cancer Research Center, UAMS, Little Rock, Arkansas, USA; [2]Scientific Direction, Istituto Clinico Humanitas, Rozzano, Milano, Italy; [3]Department of Pathology, Istituto Clinico Humanitas, Rozzano, Milan, Italy.E-mail: fabio.grizzi@humanitas.it

Dendritic cells (DC) are highly specialized antigens presenting cells present in peripheral lymphoid and non-lymphoid organs. DC are also present in the thymus, where they serve to eliminate potentially autoreactive cells from the T-cells repertoire. The major characteristic qualifying DC as professional antigen presenting cells is their high expression of MHC antigens and adhesion/co-stimulation molecules. Since both macrophages (M) and DC can differentiate from blood monocytes, depending on the different culture conditions, we investigated their kinetics of expression of MHC antigens and several adhesion/co-stimulation molecules. 42 ml of peripheral blood from three healthy volunteers was centrifuged on Ficoll-Hypaque to obtain PMBC. These cells were then plated (1x107/3 ml per well) in AIM-V culture medium. After 2 h at 37° C, non-adherent cells were removed, and the adherent cells were cultured at 37° C in a humidified 5% CO2/95 % air incubator, in medium supplemented with recombinant human GM-CSF (800 U/ml), and IL-4 (1000 U/ml). Cells were evaluated for surface marker expression using FACS analysis at different time points [i. e. time 0, day 5, day 7 and three days further in the presence of TNF-a (day 10)]. For each time point, a panel of mAbs recognizing the following antigens was used: CD 40, CD 54, CD 80, CD1a, CD 86, CD 14 and CD83. MHC Class I and MHC Class II were highly expressed in DC. CD 80, CD 86 and CD 54 expressions increased significantly until day 10, while CD 1a remained stable throughout the culture period. M cells maintained up-regulated CD 86, whereas CD1a declined throughout the culture period. CD 54 remained highly expressed in M, becoming undetectable by day 5 whereas CD 40 was transiently expressed on M until day 5. Our results show the feasibility in analyzing the different patterns in surface antigens on human DC. These qualitative and quantitative immunologically differences in respect to other cells, such as M, enable their different properties in naive T cell priming.

VT-2018

CYTOTOXIC EFFECTS OF CHEMICALLY MODIFIED TETRACYCLINES ON R22 CELLS. KIMBERLY GUILFOY, Sanford R. Simon and Elizabeth J. Roemer, Department of Pathology, SUNY Stony Brook, Stony Brook, NY 11794-8691 E-mail: kguilfoy@ic.sunysb.edu

Many cancer treatments affect not only tumor cells, but normal body cells as well. In vitro assays for potential cancer treatments are therefore used to help determine a potential medication¡s effect on both of these types of cells. Ideal treatments should show the greatest cytotoxic effects on tumor cells, while normal cells experience minimal damage. Using in vitro cytotoxicity assays on rat heart smooth muscle cells (R22) enables chemically modified tetracyclines (CMTs) to be screened for their cytotoxic effects on a ¡°normal¡± cell. The R22 cells are treated with the CMTs at both confluent and sub-confluent densities. This permits screening of both mitotic and quiescent cells, and thus roughly models representing the varying levels of mitotic activity seen in different tissue types. A cytotoxic dose response to the CMTs is the primary purpose to this particular assay. MTS, a tetrazolium salt, was used as an indicator of the CMTs cytotoxic effect. The MTS indicator, which is normally yellow, becomes brown when reduced by the viable R22 cells. Therefore, the darker the color, the greater the number of surviving cells. The optical density produced by the color change is measured, and the IC50 calculated for each of the CMTs. The IC50 provides the concentration of each CMT at which half of the cells are dead. Visual observations determined the morphological effects the CMTs had on the R22 cells. This assay; along with numerous additional cytotoxic assays; is being used to help determine which CMTs have the best potential as cancer treatments. This study was supported by NIH(NIDER) DE-10985 (431-0264A); CollaGenex Pharmaceuticals, Inc., (431-6087A); USAMRMC DA-MD-1798-18560 (431-1241A); SUSB Center for Biotechnology (NYS Science & Technology Foundation) (431-X324Q) and URECA Grant #264900.

VT-2019

Biochemical Changes Induced in Human Cells by the Vesicating Agent Sulfur Mustard. W.J. SMITH, O.E. Clark, F.M. Cowan, M.E. DeJoseph and C.L. Gross. Pharmacology Division, US Army Medical Research Institute of Chemical Defense, APG, MD 21010-5400. E-mail: WILLIAM.SMITH@AMEDD.ARMY.MIL

Development of medical countermeasures against blistering agents requires definition of the pathophysiology produced by these agents. Sulfur mustard (HD) is a genotoxic agent that causes severe skin blistering with associated inflammatory responses. We studied three pathways of cell injury: enzyme activation, intracellular ion changes, and inflammatory mediator induction. We demonstrated time- and dose-dependent activation of poly(ADP-ribose) polymerase (PARP) in HEK. At 10 mM mustard there is a significant elevation of PARP activity by 4 hours postexposure. At 100 mM, elevated PARP was detected at 2 hours postexposure. PARP activity decreased to baseline by 6 hours and then returned with a 30% elevation at 24 hours. Our routine culture conditions using passage 3 HEK showed no large changes in intracellular calcium after HD exposure, but use of early (passage 2) HEK grown on coverslips demonstrated a low level (<20%) increase in intracellular calcium within 15 minutes of HD exposure. Expression of Fc receptors by HEK was increased following HD, as was binding of the initial complement sequence component, C1q. The interleukins IL-6 and IL-1b were only seen at the highest concentration of HD, 300 mM (a highly toxic dose). IL-8 was increased in supernates of HEK exposed to 50, 100 and 300 mM. While each pathway demonstrated some HD-induced changes, neither the extent nor the timing of the changes suggests a single mechanism of cell injury.

VT-2020

Phototoxicity Testing Using 3-D Reconstructed Human Skin Models. P.A. JONES, A.V. King and L.K. Earl, SEAC Toxicology Unit, Unilever Research Colworth, Colworth House, Sharnbrook, Bedford, UK, MK44 1LQ. Email: penny.jones@unilever.com

The potential phototoxic hazard of materials is currently assessed in-house using a tiered testing strategy involving *in vitro* assays, including the validated 3T3 cell neutral red uptake phototoxicity test. However, a second test is required to allow confirmatory testing of materials of low aqueous solubility, prior to their use in products. The purpose of this study was to investigate if an *in vitro* 3-D reconstructed human skin model, EpiDerm™ (MatTek Corporation, USA) could be used to identify the phototoxic hazard of such test materials. The assay was based on the methods of Liebsch *et al* (ALTEX, **14**, 165, 1997) but with some differences. A UVB filter was used instead of a UVA filter and organic solvents were used as the dosing vehicles instead of water or sesame oil. 8 chemicals of known *in vivo* phototoxicity and 4 ingredients were assayed. EpiDerm™ cultures were treated for 18 hours with 20 microlitres test material or vehicle, then exposed to UV light (1.7mW/cm²) or dark, for 1 hour. Cultures were incubated overnight, then a MTT cytotoxicity assay was performed, with results expressed as % control MTT conversion. The testing strategy was to assess the phototoxicity of 2 concentrations at the maximum causing no significant dark toxicity (up to 100mg/ml). The criterion for phototoxicity was a 30% difference in dark/light toxicity at one concentration. This strategy minimised the number of cultures used and therefore the cost of testing. EpiDerm™ was also compared with SkinEthic™ (Laboratoire SkinEthic™, Nice, France) using light microscopy and by assessing the phototoxicity of anthracene. The EpiDerm™ assay correctly identified the known phototoxicity of the chemicals tested. The ingredients were found to be non-phototoxic, confirming previous 3T3 assay results but at more relevant concentrations. The results also indicated that both culture models could be used to detect the phototoxic hazard of anthracene and by inference other materials. However, further investigations may be needed to determine if the same order of potency of materials is given by the models compared with human *in vivo* data.

VT-2021

Recuperative Effect of Fermented Milk, Kefir to UV-Damaged Cultured Cells. K. TERUYA, T. Nagira, J. Narisawa, K. Kusumoto*, Y. Katakura, D. W. Barnes*, S. Tokumaru** and S. Shirahata. Graduate School of Genetic Resources Technology, Kyushu University, Fukuoka 812-8581, Japan; *American Type Culture Collection, Manassas VA 20110, USA; **Nihon Kefir Co. Ltd., Fujisawa 251-0054, Japan. Email: kteruya@grt.kyushu-u.ac.jp

An irradiation of ultraviolet ray (UV) produces reactive oxygen species (ROS) which cause DNA damage on chromosomes in cells. The damage results in genetic mutation, cell death and/or cellular carcinogenesis. Therefore, a search for recuperative substances to UV irradiation is important to prevent diseases caused by DNA damage such as cancer. It was found that aqueous extract of Kefir derived from Caucasus mountain in Russia exhibited scavenging activity to ROS and let abnormal oxidized status caused by UV irradiation recover into reduced state, by analysis using ROS-sensitive fluorescent dye. To investigate DNA repair enhancement by Kefir, thymine dimer formed by UV irradiation was determined by using anti-thymine dimer antibody. Kefir treatment reduced the amount of thymine dimer formed after UV irradiation. Unscheduled DNA synthesis corresponding to the DNA repair was rapidly enhanced by treatment of UV irradiated cells with Kefir extract. Kefir treatment lead UV-irradiated cells into rising of colony forming ability and suppression of apoptosis than control without Kefir treatment. Gel filtration using Sephadex G-25 revealed that the active substance in Kefir was below MW 5,000. These results suggested that the aqueous substances below MW 5,000 in Kefir recuperated from DNA damage induced by UV irradiation.

VT-2022

Effects of Chemical Inhibitors on Protein Tyrosine Phosphorylation and Cytotoxicity Shiu Moy and Wendy Scholz Nalge Nunc International Corporation, 2000 N. Aurora Road, Naperville, IL 60563. E-mail: wscholz@nalgenunc.com

Tyrosine kinases activate proteins that are involved in the signal transduction pathways leading to proliferation or oncogenesis. Thus, chemical compounds capable of altering phosphorylation of proteins might be potential antineoplastic agents. L929 cells were grown on membrane bottom plates and subjected to kinase and phosphatase inhibitors for protein phosphorylation and cytotoxicity analyses. Changes in protein phosphorylation in cell lysate were evaluated by probing with biotinylated anti-phosphotyrosine antibodies, avidin-HRP, and detecting with either TMB or luminol. In addition, tyrosine phosphorylated proteins were identified by gel electrophoresis and immunoblot analysis. Cytotoxic effects were determined using fluorescein diacetate on cells grown in black, low autofluorescent membrane bottom plates. Immunoblot analysis of proteins from L929 cell lysate separated by gel electrophoresis indicated that proteins were phosphorylated and dephosphorylated depending on the chemical inhibitors. Tyrosine phosphorylation seemed to be enhanced by Phenylarsine Oxide (PAO). A significant increase in the phosphorylation of a 116-kDa and a 150-kDa protein was observed. Among the chemical inhibitors screened for cytotoxic activities, PAO was observed to alter the morphology of L929 after 30 minutes of incubation. If phosphorylation of these proteins is linked to cell cytotoxicity, then these two proteins could be potential targets for the development of antineoplastic drugs.

VT-2023

The Spontaneously Contracting Primary Cultures of Neonatal Rat Cardiomyocytes as an In Vitro Toxicity Screening System. U. SCHRAMM, M.D. Estevez, B. Greiner, W. Frieauff, A. Wolf. Novartis Pharma AG, Toxicology/Pathology, CH-4002 Basel, Switzerland. E-mail: ursula.schramm@pharma.novartis.com

The purpose of this study was to evaluate the suitability of the spontaneously contracting primary cultures of neonatal rat cardiomyocytes as an in vitro cardiac cell system for the classification of a compound's cardiotoxic potential. The effects of known in vivo cardiotoxic compounds (amiodarone, chlorpromazine, clomipramine, doxorubicin, emetine, lidocaine, ouabain, phenobarbital, quinidine, tetraethylammonium, theophylline, verapamil), non-specific cell toxins (m-fluorotyrosine, mercury chloride) and non-cardiotoxic compounds (dexamethasone, diclofenac, kanamycin, paracetamol) were studied. Morphological, biochemical (creatine kinase (CK) leakage) and functional (frequency of contraction) parameters were evaluated. Treatment of cardiomyocytes with dexamethasone, diclofenac, kanamycin, and paracetamol had no effect on morphology, CK leakage and the frequency of contraction. M-fluorotyrosine, lidocaine, ouabaine, phenobarbital, tetraethylammonium and theophylline did not induce cytotoxic effects, but decreased the frequency of contraction. Morphological changes and a slight increase in CK leakage associated with an inhibition of contraction, were observed after treatment with quinidine and verapamil. Cellular lysis and a strong increase in CK leakage were found after incubation with amiodarone, chlorpromazine, clomipramine, doxorubicin, emetine, terfernadine, and mercury chloride even at low concentrations. In parallel a complete inhibition of the frequency of contraction was observed. After comparison with in vivo data the results suggest, that the primary culture of neonatal rat cardiomyocytes is a useful in vitro model to predict a potential myocardial toxicity of a compound.

VT-2024

Predictive Value of In Vitro Chemosensitivity Test Using the 3-Dimentional Collagen Gel Droplet Culture Method in Recurrent Non Small Lung Cancer. Hisayuki Kobayashi. Research & Development Nitta Gelatin Inc. Osaka, Japan. E-mail:LEL00157@nifty.ne.jp

Collagen gel droplet embedded culture drug sensitivity test(CD-DST) accurately identifies responders and non-responders to specific chemotherapy agents and it was able to evaluate the anticancer effect for cancer cells without contaminating fibroblast cells(Int.J.Oncol.11:449,1997). In the present study we determined whether the chemosensitivities of primary non small cell lung cancers(NSCLC) measured in CD-DST was predictive of response for recurrent tumors retrospectively. The CD-DST performed on surgical specimens from 243 NSCLS cases and 185 cases(76%) were evaluable. Drug response to cisplatin(CDDP),carboplatin(CBDCA),mitomycin C(MMC), etoposide(VP-16), 5-fluorouracil(5-FU), vindesine(VDS) and doxorubicin(DOX) was measured in exposing pharmacological dose. The in vitro sensitivity was expressed as the percentage T/C ratio, where T was the result of the treated group and C was the result of the control group; a T/C ratio of 50% or less was regarded as being sensitive in vitro. In Twenty-five patients with measurable recurrent lesionswho received platinum-based chemotherapy, the clinical responses wereCR;2, PR;6, NC;3 and PD;0 (response rate 73%) in the CD-DST responder. In contrast there was only one response(PR) in the CD-DST non-responder, others couldn't have clinical response. The comparison of the results in CD-DST and CDDP-based clinical treatment were shown good prediction(true positive rate;73%, true negative rate; 93%, accuracy; 84%). There was a statistically significant correlation in clinical response rate between the responder and non-responder ($p=0.0021$). We conclude that the chemosensitivity Information of primary NSCLC measured by CD-DST may be a useful for chemotherapy against recurrent NSCLC.

VT-2025

In Vitro Cytotoxicity of S-Nicotine and S-Cotinine as Determined by the FRAME's Neutral Red Uptake (NR) and Kenacid Blue (KB) Assays. J. OEY & E. Roemer, Institut fuer biologische Forschung, Cologne, FRG. Email: Viol.Brigitte@pmintl.ch

In vitro cytotoxicity of S-nicotine and its metabolite S-cotinine was determined towards mouse fibroblasts in monolayer culture using the FRAME's NR and KB assays. Vital staining by neutral red is based on the ability of the slightly basic dye to be absorbed into undamaged lysosomes, but since lysosomal activity may be influenced by added toxicants, the KB assay (protein determination) was also used. A difference in activity determined in the two assays was found for S-nicotine but not for S-cotinine, indicating a lysosomal effect of S-nicotine. The midpoint cytotoxicity (50 %) value for S-nicotine was 17 mmol/l in the NR assay and 10 mmol/l in the KB assay. The values for S-cotinine were 50 mmol/l in both assays. Only S-nicotine was found to induce vacuolation, a phenomenon that has been attributed to cytotoxic activity. Neutral red rapidly entered the vacuoles, indicating their lysosomal origin. Induced lysosomal swellings (vacoules) were reversible upon incubation in S-nicotine-free medium. The higher in vitro cytotoxicity seen for S-nicotine than for its metabolite S-cotinine was also seen, but to a greater degree, in acute toxicity studies in vivo, e.g., LD₅₀ values were 5.9 mg/kg for (-)-nicotine administered to mice by the i.p. route [Sofia and Knobloch, Toxicol. Appl. Pharmacol. 28:227 (1974)] and 930 mg/kg for (-)-cotinine [Borzelleca et al., J. Pharmacol. Exp. Therap. 137:313 (1962)]. Our data indicate that in vitro cytotoxicity of S-nicotine is related to an injured lysosomal activity; whereas, in vitro cytotoxicity of S-cotinine is related to general cytotoxicity. (Supported by Philip Morris, USA)

VT-2026

Induction of Secondary Light Chain Gene Recombination in Human Plasma Cells by Caffeine is Independent from both the Upregulation of RAG Proteins Expression and Germ-Line Transcription. HIROFUMI TACHIBANA, Takanori Chiwata, Yuka Nagahiro, Hirotaka Haruta, and Koji Yamada Division of Bioresources and Bioenvironmental Science, Graduate School, Kyushu University, Hakozaki 6-10-1, Higashi-ku, Fukuoka, 812-8581, Japan. E-mail: tatibana@agr.kyushu-u.ac.jp

Immunoglobulin (Ig) genes are assembled during B cell development through a series of site-specific recombination events termed V(D)J recombination. The V(D)J recombination reaction is initiated by the recombination activating genes products, RAG-1 and RAG-2 and is completed by a set of proteins employed for DNA double-stranded break repair. Although many of the recombinations at the light chain loci occur in pre-B cells, recent findings suggest that light chain gene rearrangements often continues in immature-B cells and germinal center B cells. We also have shown that secondary VJ recombination can occur in some human plasma cell lines when stimulate with concanavalin A. We call this process light chain shifting. Secondary VJ recombination in immature B cells and our plasma B cells are closely correlated with coexpression of the two RAG genes. However, there must be additional levels of regulation to determine which loci are targeted for recombination in developing lymphocytes, because endogenous Ig genes are only targeted for complete rearrangement in B cells, and endogenous T cell receptor genes are only completely rearranged in T cells. Specific developmental signals result in changes in the chromatin that allows the recombinase access to particular gene segments. Results showing that activation of a locus for rearrangement correlates with the transcription of that particular germline locus support the accessibility hypothesis. In this paper, we investigated various intracellular factors which may induce light chain shifting in human plasma cells. We found that the RAG gene products and the transcription of the germline locus is not sufficient to carry out secondary VJ recombination in human plasma cells. However, phosphatase inhibition by caffeine or okadaic acid was found to be necessary to induce secondary VJ recombination. The phosphatase inhibition induced the formation of signal broken-ended DNA intermediates in the cells, which is an initial step in the V(D)J recombination process.

VT-2027

Acute and Chronic Levels of Cytotoxicity and Immuno-stimulation for 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), a Polymer Catalyst, Using In Vitro Modeled Assays. A.M. Wright; J.G.Strom; M.E. Rothenberg. Mercer University, School of Pharmacy, Atlanta Georgia 30341. Email: wright_am@acadmn.mercer.edu

DBU is a water-soluble catalyst used in the polymerization of polymer components in the development of possible biomaterials. The acute and chronic levels of toxicity of DBU were determined using in vitro assays. The assays were Neutral Red Assay for Cytotoxicity (NR), TNF-sensitive WEHI-var13 for factor alpha and beta (TNF), and ELISA assays for TNF-alpha (ELISA). Initial NR toxicity tests were run using the L929 mouse fibroblastic cell line (ATCC CCL-1) on a 96 well plate at various concentrations and time points. The determined concentrations for an acute level and a chronic level of DBU toxicity were noted. From these determined toxic concentrations, WEHI-var13 cells actinomycin D sensitive cells (ATCC CCL 2148) were exposed for 18 hours to these concentrations to initially screen for TNF production. The IC-21 mouse macrophage cells (ATCC TIB-186) was then used to stimulate TNF from the macrophages by known toxic concentrations of DBU. These supernatants were both tested using the TNF assay and mouse specific ELISA TNF-alpha kits. These determined toxic levels and immuno-stimulation by DBU would be helpful in monitoring extractable DBU concentrations from the chemical polymer extraction procedures and in vivo biomaterial extraction.

VT-2028

Inhibitory Effect of Sialyloligosaccharide and Their Derivatives on Adhesion of Entero-Pathogenic Bacteria to Human Intestinal Epithelial Cells. Y. SUGITA-KONISHI[1], S. SAKANAKA[2], K. Sasaki[2] and F. AMANO[3]. [1] Dept. of Biomedical Food Research, [3] Dept of Biochemistry and Cell Biology of National Institute of Infectious Diseases, [2] Central Research Lab., Taiyo Kagaku Company, Ltd., E-mail: ykonishi@nih.go.jp

Purpose-The emergence and re-emergence of entero-bacteria as *Salmonella enteritidis* (S.E.), *Escherichia coli (E.coli)* and *Vibrio parahaemolyticus* (V.P) has raised the necessity to develop the useful preventive food ingredients. Adhesion to intestinal epithelial cells is essential and initial step for the infection of these entero-pathogenic bacteria. Recent studies have revealed that such carbohydrate as oligosaccharides on the surface of the host eithelial cells play an important role on adherence of these pathogenic bacteria. The aim of this study is to develop anti-adhesion therapy using natural and non-toxic oligosaccharides from egg yolk, especially sialyoligosaccharides. *Materials and Methods*- Sialyloligosaccharide peptide (SGP) was prepared from delipidated egg yolk through some ion-exchange chromatography. Sialyloligosaccharide (YDS) was prepared by glycopeptidase digestion of SGP. Asialoligosaccharide (Asialo YDS) was prepared by sialydase treatment of YDS. The pathogenic strain S.E., *E. coli* and V.P. were used in the adhesion assay to human epithelial cell lines, Caco-2 cell and T84, which had been precultured onto a 24 well plate for 10–14 days to establish a fully-differentiated epithelial layer. After incubation with various concentrations of these oligosaccharides derivatives for 30 min, the cells were infected with each bacteria at MOI 100, followed by incubation for 1 hr at 4 ¡C. After washing with PBS three time, the bacteria adhered to cells were recovered by digestion of the cells with 0.1% Triton-X in PBS and the seeded viable bacteria number was counted on TSagar plate as cfu. Oligomannose was used as positive control. *Results and Discussion*- Sialyloligosaccharide and their derivatives inhibited the adhesion of S.E and V. P. to Caco-2 cells and T84, while that of *E.coli* was not. These results show that the oligosaccharides posses an inhibitory activity on the adhesion of some entero-pathogenic bacteria, suggesting the usefulness these oligosaccharide as preventive food ingredients.

VT-2029

Age and Ocular Irritancy as Measured In Vitro. J.G. SIVAK, K.L. Moran and D.G. Dixon. School of Optometry and Department of Biology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1. E-mail: jsivak@sciborg.uwaterloo.ca

The Draize eye irritation test involves the injection of 0.1 ml of a pure chemical into the conjunctival sac of young adult rabbits. A numerical scale is used to determine if the chemical is an irritating or non-irritating substance. This is the standard eye irritancy test used today. Aside from ethical concerns, and concerns related to the subjectivity of the measures, the Draize test fails to acknowledge whether there is a change in sensitivity of the eye to irritation as the subject ages. Some chemicals, particularly cosmetics and shampoos, are used throughout a human lifespan. A rat lens model of young (2.5 month) versus older (16 month) animals were used to show that there is a change in the sensitivity to a known irritant, hexanol, with age, and that recovery (repair of incurred damage) is also age dependent. The optics of the in vitro lens, tested using a specially developed Scanning Lens Monitor, are sensitive enough to show small variations in hexanol damage with various aged lenses. Lenses from 2.5 and 16 month old rats showed 100% increases in focal length variability (optical focus), respectively, at 0.6 ± 0.7 and 10.0 ± 5.3 hours after removal from a 2 hour exposure to 100% hexanol. Recovery was seen only with the younger lenses at 46.8 ± 5.5 hours.

VT-2030

New Colorimetric Method for *In Vitro* Cell Number Estimation Using Carmine as a Specific Chromosome Dye. T. GARCIA-GASCA, V. Paz-González, M.C. Moncada-Alvarez and L.A. Salazar-Olivo. Laboratory of Toxicology In Vitro, Department of Food Research, Faculty of Chemistry, Universidad Autónoma de Querétaro, Cerro de las Campanas, Centro Universitario, 76010, Querétaro, México. E-mail: olivo@sunserver.uaq.mx

The *in vitro* animal cell culture systems are an invaluable tool for studies approaching metabolic or pharmacological effects of natural and synthetic compounds. Usually, such studies must quantify cell density in order to evaluate mitogenic or cytotoxic effects. However, estimation of *in vitro* cell density by direct cell counting is a laborious and time-consuming task. Indirect radioactive methods for cell quantitation involve health and environmental risks, whereas available colorimetric methods suffer of inaccuracies and non specific interferences. We developed a new colorimetric method for *in vitro* cell density estimation by using carmine, a natural dye widely used for chromosome staining in histological studies. The study was performed employing the established insect cell line Tn5B1-4, the human epithelioid HeLa cell line, the transformed fibroblastic murine cell lines NIH 3T3/Ha-ras and NIH 3T3/v-mos, the normal murine preadipose cell line 3T3-F442A, as well as a fibroblastic avian cell strain. Two different stain preparations were evaluated, and conditions for cell staining and fixed dye elution and quantitation were determined. Titration curves for the different cell lines showed high correlation coefficients between cell number and extracted stain, within a range of 5 x 10^3 to 5 x 10^5 cells. The proposed technique represents an useful and reliable alternative for *in vitro* cell quantitation, avoiding imprecisions derived from interferences caused by modifiable cell components. Also, the reported new method is successfully applicable to a wide range of cell types and cell densities.

VT-2031

Authentication, Amelogenin, and Sex in Human Cell Lines. YVONNE A. REID, Scott Durkin, and Debra Boles. Cell Biology Program, American Type Culture Collection, Manassas, VA 20110. Email: yreid@atcc.org

DNA fingerprinting is one of several techniques that is routinely employed by Culture Collections as part of a rigorous quality control procedure for the authentication of human cell lines. As part of the accessioning process at the ATCC, each cell line in the Master Cell Bank (MCB) is characterized by eight STR loci. The level of discrimination of the markers is approximately 1 in 109. This should prove sufficient to characterize all the human cell lines presently in the ATCC repository. Additionally, inclusion of primers for the amplification of the amelogenin locus, which allows gender identification based on differential sex chromosome specific product size, has provided important information to our holdings.

VT-2032

The Choice of Vehicle Solution for Cryoprotectants Impacts Cell Viability After Exposure at Low Temperatures. L.H. CAMPBELL, R.N. Rutledge, M.J. Taylor and K.G.M Brockbank. Organ Recovery Systems, Inc., Port City Center, Charleston, SC 29403. E-mail: lcampbell@organ-recovery.com

A variety of factors are known to influence the survival of cells during cryopreservation, but the role of the vehicle solution for the cryoprotective agents (CPAs) is often overlooked. It is generally assumed that conventional culture media used to nurture cells at physiological temperatures will also provide a suitable medium for exposure at low temperatures. However, it is now well established in tissue and organ preservation that maintenance of the ionic and hydraulic balance in cells during hypothermia can be better controlled by using solutions designed to physically restrict these temperature-induced imbalances. On this basis, we have formulated a new hypothermic preservation solution (designated UNISOL™) and have compared it with EuroCollins (EC), an established organ preservation solution that has been used previously as a vehicle solution for CPAs. Two cells types, a smooth muscle cell line and an endothelial cell line, were exposed to a range of concentrations of either dimethyl sulfoxide (DMSO) or propanediol, for 10 minutes at 4°C. Elution of the CPAs was accomplished using mannitol as an osmotic buffer at 4°C. Additional groups of cells were treated similarly except they were also frozen and thawed in the presence of the various preservation solution/CPA combinations. After rewarming at 37°C and resuspension in culture medium, all groups of cells were assessed for metabolic activity using the non-toxic indicator Alamar Blue (Trek Diagnostics). For both the frozen and non-frozen groups of cells, viability was shown to be dependent on both the concentration of CPA and the composition of the vehicle solution. Cell viability was consistently higher after treatment with Unisol compared with EC and the highest recovery (~75%) after freezing and thawing was achieved using Unisol containing 1-2M DMSO. We conclude that optimum preservation of cells during low temperature storage techniques is impacted by the nature of the vehicle solution and that Unisol offers a new alternative for improved cell survival after cryopreservation.

VT-2033

Development of Protein-Free Media for High Density Suspension Culture of HEK 293 Cells and Recombinant Adenovirus. C.J. CARD and B.B. Barnett, HyClone Labs, Inc. Logan, UT 84321. E-mail: corwin.card@perbio.com

The field of gene therapy has stimulated great enthusiasm as well as great apprehension. Extreme care must be taken to ensure the quality and purity of products used for gene therapy. Many questions are generated as the concept of gene therapy matures. These include the choice of an appropriate vector (e.g. viral, naked plasmid, liposomal), and which vehicle to produce these vectors. The HEK 293 cell line has become popular for production of viral vectors; commonly, the adenovirus vector. Once a cell line has been selected, attention must be given to the culturing of the cells, growth medium, vector production, and downstream processing of the product. A protein-free medium simplifies the manufacturing of these products by reducing concerns over adventitious agents, and facilitating downstream processing and purification. The development of a protein-free medium for the culturing of HEK 293 cells will be described. This same method could be used for the development of culture media for other cell lines. The medium produces high-density suspension growth of HEK 293 cells, as well as high yields of recombinant adenovirus.

VT-2034

The Development of Serum Free Media for Vaccine Production in Vero Cells. G. W. REESE, B. B. Barnett and C. J. Card. HyClone Laboratories, Logan, UT 84321. Email: gordon.reese@perbio.com

The development of serum-free (SF) media for attachment-dependent cells brings challenges not found with suspension culture SF media development. Most notably, serum provides attachment and spreading factors and aids in the recovery from cell disassociation procedures (e.g., trypsin treatment). For example, virus vaccine production processes require robust cell monolayers that can withstand the manipulations associated with handling large numbers of flasks or roller bottles. Therefore, SF media must provide factors supporting cell attachment, formation and maintenance of monolayers, and virus yields matching or exceeding those obtained with serum-containing media. This paper describes the development of a serum-free medium for virus production in Vero cells. The results of cell growth, virus yield and metabolic studies are presented. Time-lapse photomicroscopy demonstrates observable differences in monolayer formation between cultures with and without serum. A serum-free medium was developed that supports virus yields higher than those obtained from serum containing media.

VT-2035

Cell Growth and Differentiation on Chemically Modified Surfaces. W.K.SCHOLZ and S.K.W. Nanda. Nalge Nunc International, 2000 N. Aurora Rd, Naperville, IL 60540. E-mail:WScholz@nalgenunc.com

Growth substrates affect the adhesion, growth and differentiation of many cell types. Most cell types prefer surfaces with a high surface energy such as those modified by corona discharge. However, certain cell types, such as primary neurons, require specific interaction with functional groups provided by polylysine or biologically derived coatings such as laminin or fibronectin. We examined the attachment and morphology of primary chick brain cells on non-modified and chemically modified polystyrene surfaces. Chemical modification was accomplished by coating the growth surface with a cationic polyamine polymer. Quantitation of primary amines on the modified surface was determined by utilizing 3-(4-carboxybenzoyl)quinoline-2-carboxaldehyde, a non-fluorescent reagent that reacts with primary amines, in the presence of cyanide, to form a highly fluorescent derivative. Secondary amines on the modified surface were measured by adapting the bicinchonic acid protein assay using a water-soluble sulfo-SHPP reagent. Using these methodologies, the amount of primary and secondary amines detected on the chemically modified surface was less than on a polylysine coated surface. Microscopic examination demonstrated excellent neuronal growth and differentiation, suggesting that the surface minimally satisfied neuronal requirements for cell adhesion and differentiation without supplying a biologically derived substance that may activate various signal transduction pathways within the cells.

VT-2036

Successful Transformation of Cryopreserved Lymphocytes: A Resource for Epidemiological Studies. J.C. Beck (1), C.M. BEISWANGER (1), E. John (2), E. Satariano (2), and D. West (2). (1) Coriell Institute for Medical Research, Camden, NJ 08103 and (2) Northern California Cancer Center, Union City, CA 94587. E-mail: jbeck@umdnj.edu

The Cooperative Family Registry for Breast Cancer Studies (CFRBCS) is an international multi-site cooperative consortium of investigators who collaborate to ascertain families at risk for breast cancer. From each individual participating in the Registry, sufficient blood is collected to permit the isolation of lymphocytes, distribution into four aliquots and cryopreservation for future transformation. To date, cell lines have been established from the lymphocytes cryopreserved from 4–22 months [mean <U>+</U> SEM) = 13.9 <U>+</U> 0.2 months] from a subset of CFRBCS participants using Epstein-Barr virus transformation protocols. Cryopreserved lymphocytes from 363 CFRBCS participants were successfully transformed within 40.2 <U>+</U> 0.8 days (mean <U>+</U> SEM). However, compared to freshly isolated lymphocytes from the same study population (35.2 <U>+</U> 0.3 days), the time required for successful transformation was somewhat longer. Although the success rate for transformation was slightly less for the cryopreserved sample set (90% vs. 94% for fresh), the availability of multiple aliquots of cryopreserved lymphocytes from each Registry participant for additional transformation attempts virtually guarantees the establishment of a cell line. Cryopreservation of isolated lymphocytes represents an economic alternative to the establishment of cell lines at the time of venipuncture for epidemiological studies such as the CFRBCS.

VT-2037

Development of cell culture system from liver, kidney and spleen of African catfish Clarias gariepinus. G.S.KUMAR, I.S.B.Singh, and R.Philip*. Environmental Microbiology Laboratory, School of Environmental Studies, *School of Marine Sciences, Cochin University of Science and Technology, Fine Arts Avenue, Cochin 682 016, India, E-mail: bsingh@md3.vsnl.net.in

A viable technique for developing diploid cell cultures from liver, spleen and kidney of *Clarias gariepinus* has been developed using MEM (Eagle Salts) as the basal medium supplemented with 10% foetal bovine serum (v/v). To enhance growth rate of the cells derived from liver tissue, the above medium was further modified by incorporating 10% fish muscle extract (FME) (v/v); 10% prawn muscle extract (PME) (v/v); 0.5% ovary extract (fish) (v/v), besides the addition of 0.1mg.mL-1 each lectin and lipopolysacharides (LPS); and 0.2mg.mL-1 glucose D. For the cells generated from the spleen, the medium was modified by adding 10% FME, 10% PME, 0.5% OE, 0.5% prawn haemolymph (PHL) and 0.1mg.mL-1 each lectin and LPS and 0.2 mg.mL-1 glucose D. For the cell culture develped from kidney, the medium was modified by incorporating 10% FME, 10% PME, 0.5% OE and 0.1 mg.mL-1 each lectin and LPS. The basic protocol was developed based on the explant method, and monolayer of primary cell cultures could be routinely obtained with in a period of 10 days. For subculturing, TPVG was diluted to contain 0.05% trypsin for the cells from liver and kidney and to 0.1% trypsin for those of spleen and was employed following the conventional protocol. Trypsin content over and above the concentration stated was toxic to the cell cultures developed. During subculturing 60–80% confluence could be obtained and the diploid cell lines could be passaged 8–9 times with a time interval of 6 to 7 days between every passage. The cell lines derived above were both epitheleoid and fibroblastc.

VT-2038

Efficient Isolation and Long-Term Viability of Bovine Small Preantral Follicles In Vitro. T. ITOH, H. Abe, M. Kacchi and H. Hoshi. Research Institute for the Functional Peptides, Yamagata 990-0823, Japan. E-mail: itoh@func-p.co.jp

A mechanical isolation techniques for small preantral follicles from bovine ovaries was compared with the enzymatic isolation method. The mean number (157.0) of intact follicles per ovary isolated by the mechanical method was significantly greater than that (26.0) of follicles isolated by the enzymatic method. Also electron microscopic observation confirmed the intact ultrastructure of mechanically isolated follicles. Mechanically isolated morphologically normal follicles (MNF) were cultured for up to 30 days either in control cultures (non co-culture) or in co-culture with bovine ovary mesenchymal cells (BOM), fetal bovine skin fibroblasts (FBF) and bovine granulosa cells (BGC). In control cultures, most of the follicles degenerated and only a few MNF (1.2%) were present after 30 days in culture. In contrast, the co-cultures with BOM, FBF and BGC resulted in 50.7%, 46.6%, 21.4%, respectively, viable MNF. Trypan blue and Hoechst 33258 staining were used for a quick and sensitive assessment of oocyte and granulosa cell viability during follicle isolation and culture in vitro. After 30 days, percentages of viable follicles in co-culture with BOM (18.6%) and FBF (17.1%) were significantly greater than those of follicles in the control cultures (0%) or in co-culture with BGC (10.0%). There was a gradual increase in the average diameter of the MNF during culture. The mean diameter of the follicles increased 15.4% and 30.0% in co-culture with BOM and FBF by Day 30. In conclusion, intact small bovine preantral follicles were efficiently isolated using a mechanical method that utilizes a grating device and could be maintained for up to 30 days in the presence of mesenchymal cell co-cultures such as BOM and FBF. This in vitro culture system that supports long-term survival of bovine preantral follicles should be beneficial for studying follicle growth and development. (Supported by PROBRAIN)

VT-2039

The Culture of Rat vibrissa Cells Using Low-serum Medium. H. TAKAHASHI, and T. Ishibashi. Tsuruga Institute of Biotechnology, TOYOBO CO.,LTD. E-mail: hidekazu_takahashi@bio.toyobo.co.jp

We attempted to develop the low-serum culture method of rat vibrissa papilla cells. First, we found proliferation activities in epithelial cell extract and bovine pituitary extract. After screening hormones, we also found positive effect in insulin, transferrin, cyproterone and so on. After we optimized concentration of each factor and added serum with low concentration (1%), we made papilla cell growth medium. This medium supported the papilla cell proliferation more than normal medium containing 10% FCS. We used alkaline phosphatase activity as marker of vibrissa papilla cells, and its expression in the process of subculture. Finally, we observed the organic structure in coculture with fibrobrast, keratinocyte and vibrissa papilla cell using collagen gel method.

VT-2040

The establishment of human T-T hybridomas producing IL-4 and IFN-gamma. KEIKO KAWAMOTO, MASAYUKI NAGASE, HIROHARU KAWAHARA*, KATSUHIRO HAKAMATA** and MARI MAEDA-YAMAMOTO**, Bio-oriented Research Advanced Institution, *Kitakyushu National College of Technology, **The National Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries, 2769 Kanaya, Shizuoka 428-8501, Japan. E-mail: marimy@tea.affrc.go.jp

We established a new human fusion partner, ICLU-T, for making human T-T hybridomas which was 6-thioguanine resistant and able to grow in serum-free medium (insulin, transferrin, ethanolamine and sodium selenite supplemented ERDF meidum) very quickly. ICLU-T was fused with peripheral blood lymphocytes of healthy volunteer by using 1% lecithin with polyethylene glycol to increase cell viability after cell fusion. The obtained T-T hybridomas chould proliferate in serum free medium. By flow cytometric analysis, the T-T hybridomas were heterogenous populations which showed CD4+CD8-, CD4-CD8+, and CD4-CD8- T cells, respectively. Neither CD14 nor CD19 expression was detected on these cells. Furthermore, immunostaining of intracellular cytokines of T-T hybridomas by using specific antibodies revealed that they produced IL-4 and/or IFN-gamma spontaneously, suggesting that these T-T hybridomas may be consisted of different sets of T cells such as helper T cells, cytotoxic T cells, and NKT cells. The further characterization of this cell line is now investigating. However, by critical cloning, it may be possible to get these phenotypes of T cells from this heterogenous populations. We suggetsed that these T-T hybridomas may be useful for screening assay system of immune regulatory factors in food. ★@*This work was supported by grants from program for promotion of basic research activities for innovative biosciences of Japan.

VT-2041

Inhibition of Calpain Increases Cell Migration and Wound Healing via a Protein Kinase C Dependent Pathway in Human Dermal Microvascular Endothelial Cells. L.L. CHIU and M.A. Karasek. Dept. of Dermatology, Stanford Medical School, Palo Alto, CA 94305. E-mail: LYNNCHIU@LELAND.STANFORD.EDU

Calpain, a calcium-dependent protease, regulates cell migration via actin remodeling. Inhibitors of calpain disrupt the connection between molecules that comprise the integrin-cytoskeletal linkage at the trailing edge of the cell and prevent migration. The purpose of this investigation was to determine if calpain is involved in the control of the transition of human dermal microvasular endothelial cells (HDMEC) from an epithelioid morphology to that of a spindle-shaped morphology central to wound healing, angiogenesis, and inflammation. To permit this transition to take place, actin filaments in HDMEC rearrange from a circular array to a parallel array. When treated with an inhibitor calpain an increase in the transition from epithelioid morphology to a spindle-shaped morphology is observed. Wound healing determined by scratch assays of confluent cultures of HDMEC is increased by inhibition of calpain. The inhibition of calpain and the conversion to a spindle-shaped morphology are reversed with Staurosporin, a powerful inhibitor of protein kinase C. These results demonstrate that a calcium-dependent protease is involved in maintaining the structural integrity of the epithelioid morphology of HDMEC, that the activity of PKC in inducing a transition is reversed by calpain inhibitors, and that calpain may play a role in the regulation of angiogenesis and wound healing.

VT-2042

Morphological Survival and In Vitro Maturation of Immature Bovine Oocytes Exposed to EGTA with or without Cryopreservation. M.R. BLANCO, L. Simonetti and P. Palermo.Facultad de Ciencias Agrarias. Universidad Nacional de Lomas de Zamora. Ruta 4 Km. 2 (1836), Llavallol, Buenos Aires, Argentina. Email: AGRARIAS@UNLZ.EDU.AR

Immature bovine oocytes are sensitive to cryopreservation procedures. EGTA may stabilize oocyte cytoskeletal and make plasma membrane less rigid to avoid injury during the osmotic stress of cryopreservation. In this study morphological survival and *in vitro* maturation (IVM) of immature bovine oocytes exposed to EGTA prior to cryoprotectant (CPA) treatment with or without cryopreservation were evaluated. Oocytes were obtained from follicles (2–6 mm) of abattoir-recovered ovaries and assigned to groups: I (Control; n=100); II (EGTA/CPA; n=98); III (CPA; n=123); IV (EGTA/CPA/ cryopreserved; n=164); V (CPA/cryopreserved; n=179). Exposition to 1mM EGTA was performed for 5 min. CPA was 1.5 M ethyleneglycol (EG) plus 10% FBS (fetal bovine serum), which was added/removed in a three-step way at 24°C for 15 min. Oocytes to be frozen were subjected to conventional cryopreservation. After thawing, oocytes were IVM (M199-HEPES-FBS-sodium piruvate-FSH-17beta estradiol-hCG) for 24 h at 39°C in 5% CO₂ in air. Then, cumulus cells were removed and morphology was assessed by stereoscopic examination. Normal morphology (NM) of ova was defined by observation of dark evenly granulated cytoplasm. Irregular shaped ova or uneven or contracted cytoplasm were considered degenerate. Oocytes having NM were fixed, stained with aceto-orcein and observed under phase-contrast microscope. Oocytes having a first polar body (FPB) and a metaphase II plate (MII) were considered IVM. Data were analyzed by ANOVA and expressed as percentages (mean±SD). NM for groups I (82.1±6.6), II (75.0±5.0) and III (78.6±10.6) was > IV (36.0±13.5) and V (48.4±13.8) ($P<0.05$). IVM for group I (93.7±7.5) was > the others, while II (81.8±5.0) and III (81.1±10.7) were > IV (65.6±4.0) and V (55.4±4.5) ($P<0.05$). In conclusion, detrimental effects of cryopreservation procedures were not improved by 1mM EGTA.

VT-2043

The relationships between thrombin-induced nitric oxide production and intracellular calcium concentrations in bovine endothelial cells. Y. MINAI, N. Takadera, M. Furusho, A. Higa, T. Hidaka, Y. Matsuoka, and M. Haga. Department of Agricultural Chemistry, Tamagawa University, Japan. Email: yminai@agr.tamagawa.ac.jp

Nitric oxide (NO) was produced constitutively by the vascular endothelial cells. Furthermore, the quantity of NO production from these endothelial cells that were stimulated with the agonist such as thrombin was higher. To aim at making a NO production mechanism by thrombin stimulus clear, the cultured bovine aotic endothelial cells were used in this research. We examined about the change in the cytoplasmic calcium concentration of the endothelial cells and about the production of NO from that cells stimulated with the thrombin. Using the cultured endothelial cells that were grown to confluent, the amount of NO secretion in the supernatant was measured by the griess reagent. The intracellular calcium concentrations were measured by the loading fluo-3AM method.First, we examined the effect of exogenous calcium ion on NO production and on the rise of intracellular calcium concentration. As compared with the absence of extracellular calcium ion, the thrombin-induced elevation of cytoplasmic calcium ion is high, however the thrombin-induced NO production is low in the presence of extracellular calcium ion. Second, we examined the effect of some signal transduction inhibitors on NO production from and intracellular calcium concentration of these endothelial cells that were stimulated with thrombin. When neomycin, phospholipase C inhibitor, was applied, the elevations of intracellular calcium concentration were suppressed and NO production was enhanced. As to the application of W7 which is calmodulin inhibitor, the NO production from the endothelial cells was also upregulated by the thrombin stimulation.These results suggested that the elevation of cytoplasmic calcium concentration could act as an inhibitory effect on NO production by thrombin stimulus.

VT-2044

The Effects of Multiple Forms of Vanadate on Sugar Transport in Human Fibroblasts. R. J. GERMINARIO, S.P. Colby-Germinario, K. Nahm, and B. Posner. Lady Davis Institute, SMBD-Jewish General Hospital, McGill University, Montreal, Que. H3T 1E2. Email: mdrl@musica.mcgill.ca

We have investigated the effects of multiple forms of vanadate on basal and insulin-stimulated sugar transport in cultured human fibroblasts(HF). ^{3}H-2 deoxy D-glucose was employed as the transportable substrate. The different forms of vanadate studied include: ortho-vanadate (VO_4), peroxide generated pervanadate (pVO_4), and 2 stable peroxovanadium compounds (Posner, B. et al, J. Biol. Chem. 269:4596–4604:1994) bpV(phen) (pVA) and bpV(pic)(pVI). After 30 min exposure, the effect of different concentrations of VO_4 on 2-DG transport exhibited a maximum effect at a concentration of 10^{-5}M with a maximal average Insulin:Control(I:C) ratio of 2.02±0.1. This I:C ratio was similar to the optimal concentrations observed for perV04; pVA and pVI. For all compounds tested, the effect on sugar transport was maximal after 30 min exposure. In a series of studies, maximal stimulating concentrations of insulin(667 nM) when combined with a maximal dose(10^{-5} M) of VO_4, per VO_4, pVA, or pVI, generated the following: the maximal I:C ratio for insulin or the various forms of vanadate alone and any insulin:vanadate combination were not significantly different from one another though all were significantly different from basal sugar transport (One Way ANOVA $P<0.01$; Tukey Analysis $P<0.05$ n=4–7). The data indicate that the effect of different vanadates affect sugar transport similarly regarding their optimally effective concentration, time exposure for response and interaction with insulin.

VT-2045

Demonstration of membrane-bound carbonic anhydrase II in human cancerous pancreatic duct cells (CAPAN-1 line). L. ALVAREZ, M. Fanjul and E. Hollande. Laboratoire de Biologie Cellulaire et Moléculaire des Epithéliums, Université Paul Sabatier, F-31400 Toulouse. Email: Etienne.HOLLANDE@wanadoo.fr

Carbonic anhydrase II (CA II) is now recognized as a cytosolic enzyme that plays a role in the synthesis of HCO_3^- ions. In pancreatic duct cells, it is involved in 25% of the production HCO_3^- of ions secreted into the lumen of the ducts, the remaining 75% being transported from the blood compartment via a basolateral Na^+/HCO_3^- exchanger. Some recent immunocytochemical studies have demonstrated the presence of membrane-bound CA II in certain tissues, but without mention of their possible role In the present study, we investigated the subcellular distribution of CA II in CAPAN-1 cells maintained in culture, by immunocytochemistry and immunoblotting of purified fractions of plasma membranes. Observation by confocal microscopy of immunocytochemical reactions on live cells or on fixed cells showed that CA II were localized in the apical plasma membranes and in cell compartments such as the nuclear envelope, the Golgi apparatus and vesicles. As a function of the stage of polarization of the cells, they were seen in perinuclear regions, throughout the cytoplasm or in cytoplasm underneath the apical membranes. When the cells were fully polarized, CA II immunoreactivity was observed in the microvilli. Immunoblots of fractions of plasma membranes purified in low ionic strength medium also demonstrated the presence of membrane-bound CA II. CA II immunoreactivity was not seen in membrane fractions purified under conditions of high ionic strength suggesting that the CA II are weakly bound to the membranes. Blockade of intracellular traffic with brefeldin A, indicated that CA II are targeted to the plasma membrane via the Golgi apparatus. The demonstration of CA II in Golgi compartments, vesicles and apical membranes suggests that this enzyme plays a role in the regulation of intra- and extracytoplasmic pH.

VT-2046

Measurement of Immune Response In Mice Coinfected With Babesia Microti And Borrelia Burgdorferi Using Enzyme Linked Immunoflourescence Assay and Immunoflourescence Assay. CHARLES D. THILL, Dreania LiVine, Jorge L. Benach. Department of Molcular Genetics and Microbiology. State University of New York at Stony Brook 11794-8691. Cthill@path.SOM.SUNYSB.edu

Babesia microti, a hemoprotozoan that causes hemolytic anemia and thrombocytopenia, and Borrelia burgdorferi, a spirochete that causes Lyme disease, are both transmitted by the same tick, Ixodes scapularis. There is evidence that both babesiosis and Lyme disease can be simultaneously induced by a single tick bite. It is, however, unclear whether one infection contributes to the severity of the other. This study was undertaken to measure several parameters of host response to simultaneous infection with B. microti and B. burgdorferi. Normal, splenectomized and aged C3H/Hen mice were inoculated intradermally with B. burgdorferi on the same day that B. microti were inoculated intraperitoneally. Single and simultaneous infections were induced in all three categories of host. Parasitemia, CBC, liver function, and presence of spirochetes were measured at appropriate time points. Antibody levels to Borrelia and Babesia were measured for both single and double infection. Borrelia antibodies were measured on ELISA plates coated with antigen prepared from Borrelia grown in stock cultures. Babesia infection was measured using indirect immunofluorescence (IFA). Infected organs were removed and placed in culture containing media and conditions suitable for growth of Borrelia. Our results indicate that both infections occur and cause disease independent of each other. Babesiosis was no worse in mice infected with Borrelia, than in mice that had this organism alone. IFA performed on serum showed no significant difference in titers between double and single infection. Likewise, the manifestations of Lyme disease and recovery of Borrelia in culture were not affected by double infections. Serologic values revealed no difference between the single and double infection. It is concluded that despite the high probability that these two pathogens can be transmitted together, the diseases that they cause have no synergy with each other.

VT-2047

Effective Cell-Free Translation System Based on 5'NCR of L-A Virus mRNA. T.EBIHARA, E. Kobatake and M.Aizawa, Department of Biological Information, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 226-8501, Japan, E-mail: tebihara@bio.titech.ac.jp

The cap structure at 5'-terminus of mRNA has been regarded as essential for the initiation of the eukaryote translation. The researches, however, have recently suggested a possible involvement of the cap independent initiation mechanisms. For example, poly(A) tail at 3'-terminus of mRNA promotes the initiation, and internal ribosomal entry site (IRES) decides the position of initiation on translation reaction due to the highly structured form from the long sequence of 5'-noncoding region (NCR). The mRNAs encoding these sequences are translated more efficiently than the mRNAs without these sequences. L-A virus is a dsRNA virus, which exists in cytosol of S.cerevisiae. Although its mRNA contains neither cap structure nor poly(A)tail, an efficient translation is performed to keep a high copy number in a single cell. So, we focused on 5'NCR of mRNA of L-A virus and linked it with a reporter gene. The mRNA has neither cap structure nor poly(A)tail. But the mRNA coding 5'NCR of L-A virus mRNA was translated more efficiently than the control mRNA. The result indicates a possible involvement of a novel translation initiation mechanism in S.cerevisiae, and the 5'NCR was utilized for effective cell-free protein synthesis system. The highly effective mRNA for translation was designed with insertion of 5'NCR from L-A virus mRNA and modification of cap structure and poly(A) tail. The ribosome was recruited efficiently onto the mRNA via three pathways of translation initiation; through cap structure, poly(A) tail, and 5'NCR of L-A virus mRNA. On the other hand, the translation condition was optimized for effective cell-free protein synthesis. As results, a highly effective yeast cell-free translation system was constructed on the basis of 5'NCR of L-A virus mRNA.

VT-2048

Developmental Changes in Expression of beta-Adrenergic Receptors in Cultures of C2C12 Skeletal Muscle Cells. R.B. YOUNG, K.Y. Bridge and J.R. Vaughn. NASA, Marshall Space Flight Center, Huntsville, AL 35812. ronald.young@msfc.nasa.gov

beta-Adrenergic receptor (bAR) agonists regulate muscle growth in several mammalian and avian species, and bAR agonists presumably exert their physiological action on skeletal muscle cells through this receptor. Because of the importance of bAR regulation on muscle protein metabolism in muscle cells, the objectives of this study were to determine the developmental expression pattern of the bAR population in C2C12 skeletal muscle cells, and to analyze changes in both the quantity and isoform expression of the major muscle protein, myosin. The number of bAR in mononucleated C2C12 cells was approximately 8,000 bAR per cell, which is comparable with the population reported in several other non-muscle cell types. However, the bAR population increased after myoblast fusion to greater than 50,000 bAR per muscle cell equivalent. The reasons for this apparent over-expression of bAR in C2C12 cells is not known. The quantity of myosin also increased after C2C12 myoblast fusion, but the quantity of myosin was less than that observed in primary muscle cell cultures. Finally, at least five different isoforms of myosin heavy chain could be resolved in C2C12 cells, and three of these exhibited either increased or decreased developmental regulation relative to the others. Thus, C2C12 myoblasts undergo developmental regulation of bAR population and a rearrangement in myosin heavy chain isoform expression.

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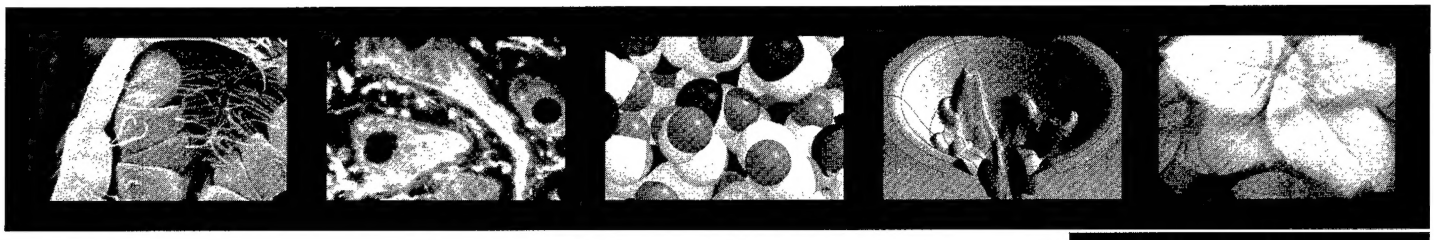

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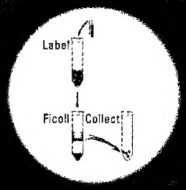

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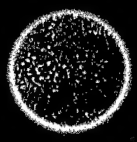

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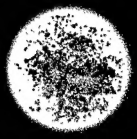

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